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CONTENTS

Ralph Eliot Smith, 1874-1953 — M. W. Gardner and J. B. Kendrick, Sr. 59
Purification and properties of tobacco ringspot virus — Russell L. Steere 60
The identity of barn rots of flue-cured tobacco in South Carolina — Quintin L. Holdeman and Walter H. Burkholder 69
Beetle transmission, host range, and properties of squash mosaic virus — J. H. Freitag 73
The validity of binomials in the leaf rust fungus complex of cereals and grasses — George B. Cummins and Ralph M. Caldwell 81
Physiological aspects of Verticillium wilt — R. P. Scheffer, S. S. Gothoskar, C. F. Pierson, and R. P. Collins 83
Effect of some soil factors on efficiency of fungicides in controlling Rhizoctonia solani — M. Rushdi and W. F. Jeffers 88
The influence of seed treatment and planting rate on the emergence and yield of soybeans — Kirk L. Athow and Ralph M. Caldwell 91
Methods of inoculation for wildfire of soybean and the effect of bacterial pustule on wildfire development — Donald W. Chamberlain 96
Resistance in pepper to Meloidogyne incognita acrita — W. W. Hare 98
Increasing the absorption of streptomycin by leaves and flowers with glycerol — Reed A. Gray 105
Control of the Helminthosporium blight diseases on sweet corn in south Florida — R. S. Cox 112
Kernel smut of big bluestem — John Dunleavy 116
The influence of nitrite on the development of Phytophthora root rot of avocado — George A. Zentmyer and Frank T. Bingham 121
Altering disease resistance with ionizing radiation — Paul E. Waggoner and A. E. Dimond 125

Phytopathological Notes:

Probable coidentity of Shiro line pattern virus and apple mosaic virus — R. M. Gilmer 127
The effect of the tobacco stunt nematode on the incidence of Fusarium wilt in flue-cured tobacco — Quintin L. Holdeman 129
Transmission to Amelanchier of an agent causing a disorder on apple — D. E. Millikan and H. W. Guengerich 130

News

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RALPH ELIOT SMITH, 1874-1953

M. W. Gardner and J. B. Kendrick, Sr.

Ralph Eliot Smith, Professor Emeritus of Plant Pathology in the University of California, died in Berkeley on December 15, 1953. He established here one of the first university departments of plant pathology and organized the agricultural experiment station research on plant diseases. He was a charter member of The American Phytopathological Society, the first president of its Pacific Division, and an associate editor of PHYTOPATHOLOGY, volumes 1 and 2.

Professor Smith was born in Boston, January 9, 1874. After graduating from Massachusetts Agricultural College in 1894, he remained there until 1903 as Assistant Professor of Botany and German, and Assistant Botanist in the Experiment Station, meanwhile spending one year with Hartig and von Tubeuf in Germany and two summers with Farlow and Seymour at Harvard. As assistant to Dr. G. E. Stone in the experiment station, he worked on many plant diseases including the aster yellows virus disease, which he was the first to describe, and asparagus rust, a disease that also was important in California.

His rust work led indirectly to an invitation from Professor Hilgard to come to California to accept an appointment as Assistant Professor of Plant Pathology at the University. He arrived on April 1, 1903, and within a few days had obtained research funds from the asparagus canners and was in the asparagus fields studying the rust.

Following a disastrous pear blight outbreak, he helped draft a bill passed by the Legislature of 1905 providing funds to the University for a campaign against blight, which with the help of Dr. M. B. Waite he organized and directed. The same legislature appropriated funds to establish a plant pathology "laboratory at Whittier with a branch at Riverside" of which he was superintendent for six years. He organized and offered courses in plant pathology at Berkeley, beginning in 1904, and was promoted to Professor in 1909.

He investigated pear scab, lemon brown rot, peach blight, olive knot, walnut blight, celery blight, tomato diseases, and curly top of sugar beets. His zeal for research was coupled always with a desire to help the farmer. He showed how to control asparagus rust by dusting with sulphur, lemon rot by adding a disinfectant to the wash water, and peach blight with an early winter spray. One is amazed at his prodigious activity. His field work ranged from the pear districts in the north to the citrus and walnut groves in the south.

Always keenly interested in walnuts, he published in 1912 a comprehensive treatise on walnut culture, varieties, and diseases. In 1912-13, he studied walnut and citrus culture in southern Europe and went on an expedition to the date gardens in the Sahara desert. The period of 1918-20 he spent in southern California developing a dust control for the walnut aphid.

RALPH ELIOT SMITH
1874—1953

This "was a major discovery in the field of insect toxicology" according to his colleague, Professor E. O. Essig.

During the next decade, Professor Smith greatly expanded his department at Berkeley and Davis. Besides research and extension, he fostered graduate study and offered an advanced course until he retired. His research was on fig diseases, peach rust, sour sap, apricot green rot, diamond canker of prune, and the gray mold fungus, Botrytis. He was specially interested in puzzling diseases of obscure or nonparasitic origin. One of his last projects was an intensive study of the fatal black-line disease or graft union failure in bearing walnut trees. Perhaps no other person was as familiar with the orchard problems of California.

Among his numerous publications (about 65) was a series of comprehensive bulletins on the plant diseases of the state. He was an able writer. In his later years he searched the early records and traced the history and study of plant diseases in California.

His sister, Elizabeth H. Smith, was his research assistant from 1905 until her death in 1933. He married Miss Jessie Anna Carroll at Whittier in 1906. They had one son, Carroll, who died at the age of fifteen. Mrs. Smith died in 1949.

In 1934, his alma mater conferred on him an honorary doctorate. He relinquished administrative work in 1936 and became professor emeritus in 1944. He was guest of honor at a dinner with a hundred of his friends in April, 1953, celebrating the fiftieth anniversary of the department he founded.

PHYTOPATHOLOGY for January (46: 1-58) was issued January 28, 1956

PURIFICATION AND PROPERTIES OF TOBACCO RINGSPOT VIRUS [1]

Russell L. Steere

SUMMARY

The infectious agent of tobacco ringspot virus was concentrated and purified from the juice of infected tobacco, petunia, squash, and cowpea plants. The purification procedure involved emulsification of 1 volume of infectious juice with 2 volumes of a cold 1:1 mixture of n-butanol and chloroform; separation of the emulsion into 3 layers by centrifugation; removal and storage of the top layer at 22°C for 12–16 hours; and finally 3 cycles of differential centrifugation. Purified preparations from all 4 hosts consisted of suspensions of uniform polyhedral particles that often appeared 6-sided in electron micrographs of frozen dried samples. The average diameter of these particles was 26 mμ, and measurements of the best hexagonal particles gave a diameter between opposite faces of 24 mμ and between opposite points of 28 mμ. Crystals were obtained by storing at 4°C a suspension of 20 mg or more virus per ml in 0.1 or 0.01 M phosphate buffer of pH 7 or in 0.01 N NaCl.

Most of the purified preparations were found to contain 2 components when analyzed with the ultracentrifuge. One of these had a sedimentation constant of 116 S, accounted for between 95 and 99 per cent of the total solid material in the preparations, and was associated with infectivity. The other, which accounted for the remaining 5 per cent or less, had a sedimentation constant of 89 S and was not associated with infectivity. The electrophoretic mobility of a 2-component sample in 0.05 M phosphate buffer at pH 7 was found to be 11.3 $\times 10^{-5}$ cm/sec/volt/cm and revealed no separation of components during 5 hours and 15 minutes.

Tobacco ringspot virus was found to contain 34.4 per cent nucleic acid. Infectivity studies following storage at 4°C verified the early findings of Stanley that 0.01 M phosphate buffer of pH 7 is a better storage medium than either distilled water or a more concentrated phosphate buffer. As much as 45 mg of purified virus was obtained from a liter of juice from infected cotyledons of Caserta squash plants. The virus was infectious at a concentration of 0.005 mg per ml and was capable of inducing a local lesion for every 120,000 particles in the inoculum. When juice from healthy plants was subjected to the purification procedure, no protein fraction was obtained.

The procedure described herein was used for purification of tobacco mosaic, tomato bushy stunt, southern bean mosaic, and turnip yellow mosaic viruses. The resultant preparations of each were monodisperse by electron microscopy and were infectious.

Preparations of centrifugally concentrated and partially purified tobacco ringspot virus (*Annulus tabaci* Holmes) (RSV) were reported by Stanley (15) to contain approximately 40 per cent nucleic acid. Among the other plant viruses that have been purified and examined chemically, only 2 (southern bean mosaic virus with 21 per cent (9) and turnip yellow mosaic virus with 35 per cent (10)) have been reported to contain more than 20 per cent nucleic acid.

Pirie (11) questioned the purity of Stanley's RSV preparations and the reported nucleic acid content thereof. He reported the isolation from healthy tobacco leaves, by use of procedures similar to those employed by Stanley for RSV purification, of considerable nucleoprotein containing a large percentage of nucleic acid.

In view of the reported high nucleic acid content of RSV, the questioned reliability of the value given, and the absence of reports of recent studies with purified RSV, it was considered appropriate to attempt to develop a procedure that would permit rapid preparation of purified suspensions of the infectious agent of this virus. Such a purification procedure would make practical more critical studies of the physical and chemical properties of RSV.

Since the initiation of the experimental work reported herein, 2 reports of other studies on the purification of RSV have appeared (8, 14). In neither of these reports was any evidence presented of the degree of homogeneity of the final preparation. The electron micrograph in the more recent of these reports (8) does show the presence of particles of rather uniform size, but it is not evident from this micrograph that the virus suspension was monodisperse.

In the initial phases of the present work, many attempts were made to obtain homogeneous suspensions of the characteristic particles of RSV by use of numerous procedures including that reported by Stanley (15). These early attempts proved fruitless. Also, juice from healthy plants when subjected to these same procedures invariably yielded pellets indistin-

[1] Accepted for publication August 15, 1955.

Aided by grants from the National Foundation for Infantile Paralysis and from the National Cancer Institute of the National Institutes of Health, Public Health Service.

It is with pleasure that I acknowledge the cooperation of Dr. R. C. Williams who gave helpful suggestions, encouragement, and assistance; of Dr. C. A. Knight who conducted the chemical assays; of Dr. H. K. Schachman who had the necessary samples analyzed by ultracentrifugation and electrophoresis; and of Dr. R. C. Backus who assisted in the microcapsule centrifugation study. I wish to acknowledge the excellent technical assistance of Mr. K. A. Miller and Mrs. Winnifred Elliott who did much of the routine work required for this study.

guishable in size and appearance from those obtained when RSV-infected plants were used.

Since the use of various organic solvents (butanol, ether, and chloroform) in procedures for the purification of other viruses had been reported (1, 7, 12),[2] numerous tests were conducted to find some organic solvent system that could be used to advantage in the purification of RSV. The purpose of this paper is to describe such a method in detail and to report methods used to obtain starting material high in virus content. Evidence of the homogeneity of the final suspensions and information concerning the infectivity, morphology, nucleic acid content, and the formation of crystalline aggregates of the characteristic particles will be presented. Preliminary reports of part of the investigations discussed here have been presented elsewhere (16, 19, 20).

BIOLOGICAL ASSAY.—Plants of black-eye cowpea (*Vigna sinensis* Endl. var. Early Ramshorn) were used for assays of the relative activities of suspensions of RSV. Primary leaves were used during the period of rapid expansion just before the growing tip of the plant began to expand. For preliminary estimates of virus concentration, the infectivities of 2 virus preparations usually were compared on opposite primary leaves. Whenever more precise measurements were desired, opposite half leaves were inoculated. For these assays, the right half of each primary leaf of ½ of the test plants was inoculated with 1 preparation; the corresponding left halves were then inoculated with the second preparation. The remaining plants were similarly inoculated; but with the 2 preparations reversed. Preliminary inoculations usually were made to determine the relative infectivities of the 2 samples that were to be compared. Each sample was then diluted to a concentration calculated to produce 20–40 lesions per half-leaf. All dilutions for assays were made with 0.01 M phosphate buffer of pH 7. Fine Carborundum (600-mesh) was added to the inoculum, and care was taken to conduct all inoculations with uniformity. Rinsing of leaves subsequent to inoculation did not affect lesion formation significantly and was generally omitted.

SELECTION OF A PRODUCTION HOST FOR RSV.—A number of different plants were grown, inoculated with RSV, and sampled for local-lesion assays of virus content. Included in this study were plants of petunia (*Petunia hybrida*); jimson weed (*Datura stramonium* L.); cucumber (*Cucumis sativus* L.); watermelon (*Citrullus vulgaris* Schrad.); flowering tobacco (*Nicotiana alata* Link & Otto); pepper (*Capsicum annuum* L.); tomato (*Lycopersicum esculentum* Mill.); tobacco (*Nicotiana tabacum* L.); and 8 different horticultural varieties of squash (*Cucurbita pepo* L.; *C. moschata* Duchesne; and *C. maxima* Duchesne). From symptom expression and from local-lesion assays, it appeared that plants of either of the 2 petunia varieties tested (Fire Chief and Snowstorm) or of one of the squashes (*Cucurbita pepo* L. var. Caserta) would be suitable hosts for the anticipated studies. Diluted juice from infected plants of these 3 hosts consistently gave 10–100 times as many lesions as did corresponding dilutions of juice from the most infectious parts of Turkish tobacco plants. Of these 3 hosts, Caserta squash was selected for use in developing the purification procedure because of the rapidity with which large quantities of infectious juice could be obtained.

CULTURE AND INOCULATION OF HOST PLANTS.—Either the seeds or young seedlings of all source or assay plants were planted in 4-in. pots filled with a rich soil. When the plants attained the proper size, the upper surface of each cotyledon or true leaf was rubbed with a cheesecloth pad saturated with inoculum. The inoculum for virus production consisted of diluted juice from RSV-infected Turkish tobacco leaves that showed acute symptoms of the disease.[3] One volume of crude juice was diluted with 9 volumes of 0.01 M phosphate buffer of pH 7, and fine Carborundum (600-mesh) was added immediately before inoculation. It was necessary to use clean inoculating pads and separate batches of inoculum for each set of 300–400 plants. Also, when squash or petunia plants were employed, it was important that they be shaded for 48 hours following inoculation. A single layer of newspapers supported above the plants was satisfactory for this purpose. Failure to observe either of these 2 conditions resulted in a reduction in the size or number of local lesions and a marked reduction in the number of plants that became systemically infected.

When a suitable procedure was finally developed, purified samples of RSV were obtained from 4 different host plants. Special requirements for culture, inoculation, or harvest are presented, therefore, for each of these.

Caserta squash.—Cotyledons were inoculated when the first true leaf was about ¾ in. in diameter. The infected cotyledons, which were found to be superior to systemically infected leaves as sources of RSV, were harvested when they began to collapse 7–12 days later.

Turkish tobacco.—Plants were inoculated when 4–6 in. high. Leaves that showed acute symptoms were harvested 3–4 weeks later for virus extraction.

Petunia.—Plants were inoculated at the 8- to 10-leaf stage, and all branches showing systemic symptoms were harvested 3–4 weeks after inoculation.

Black-eye cowpeas.—Primary leaves were inoculated during the period of rapid expansion and were harvested after they had turned brown but before they became dry.

[2] A paper by Hotchin (Hotchin, J. E. 1954. The purification and electron microscopical examination of the structure of staphylococcal bacteriophage K. Jour. Gen. Microbiol. 10: 250-260) recently has been called to the author's attention. It describes a procedure very similar to that described here. A mixture of isobutanol and chloroform was used in the purification of a bacteriophage.

[3] The original inoculum used for this work was kindly supplied in the form of "recovered leaves" by Dr. W. C. Price.

CONTROLS. — Controls consisted of healthy plants that were grown at the same time and under the same conditions as the infected plants. They were rubbed with cheesecloth pads saturated with diluted juice from healthy tobacco leaves and usually were harvested at the same time as the infected material and subjected to the same purification procedure. It was presumed, however, that the physiological condition and consequently some of the noninfectious particulates of infected plants might be more nearly like those of very young or very old healthy plants than like those of healthy plants of the same age. Therefore, samples of young and old control material were harvested and subjected to the purification procedure to serve as additional controls.

PURIFICATION PROCEDURE.—Infected leaves showing severe symptoms were ground in a meat grinder or a mechanical salad chopper, and the juice was extracted mechanically or by manually squeezing through 2 layers of cheesecloth. The virus was then extracted from the juice and purified by the procedure outlined in Figure 1 and described below. Two volumes of a 1:1 mixture of n-butanol and chloroform at a temperature of 4°C were added slowly to 1 volume of crude juice while the juice was rapidly stirred. The resulting emulsion was stirred for an additional 15 minutes, then separated into 3 layers by centrifuging for ½ hour at approximately 1000 G. The bottom layer consisted of a mixture of n-butanol and chloroform and contained all of the chlorophyll. Above this was a solid layer consisting of denatured protein and cellular debris. The top layer consisted of an aqueous phase saturated with butanol and chloroform and contained nearly all of the virus and much noninfectious protein. This aqueous phase was removed from the top of each tube, combined with that from other tubes, covered, and stored 12–16 hours at 22°C. During this period, most of the nonvirus protein was denatured and settled to the bottom of the container. The suspension was then clarified by centrifuging for 30 minutes at 8000 rpm in a Servall centrifuge, type SS-1. The clear amber liquid that remained contained most of the activity present in the original crude juice. This clarified aqueous suspension was centrifuged for 2 hours at 30,000 rpm (78,000 G) to pellet the virus. The supernatant liquid was discarded and the centrifuge tubes drained. All pellets were covered with distilled water and allowed to stand 2–3 hours at 4°C. The covering liquid was then caused to flow over the pellets to resuspend the small portions remaining. Noninfectious denatured protein was removed from this aqueous suspension by centrifuging for 15 minutes at 1000 G. To eliminate additional contaminants, 2 more cycles of differential centrifugation were employed. Each of these consisted of a 30-minute centrifugation at 40,000 rpm (105,000 G), resuspension of the virus in distilled water, then centrifugation of the virus suspension for 15 minutes at 1000 G to remove contaminants.

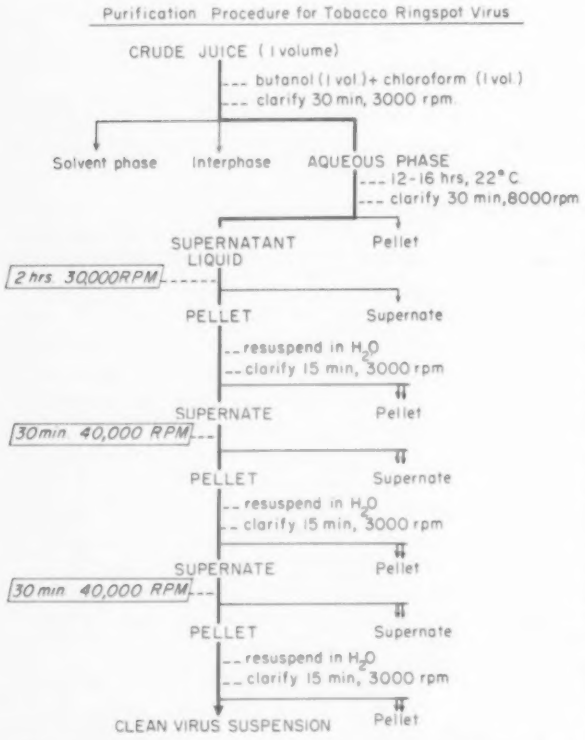

FIG. 1. Flow diagram for tobacco ringspot virus purification. Heavy line denotes fractions that were retained. Small single arrows denote fractions that were discarded, and double arrows denote fractions from which additional virus could be recovered.

Except in special cases, the suspensions were diluted with 0.01 M phosphate buffer of pH 7 after each resuspension of the pellets from high-speed centrifugation. The pellets resuspend rapidly in distilled water but very slowly in the buffer. The virus appeared to be more stable when buffered, however, and was stored in this condition whenever possible. The pellets obtained from the second 30-minute centrifugation were often colorless when RSV-infected squash cotyledons or primary leaves of black-eye cowpea were the starting material. On the other hand, when RSV-infected petunia or tobacco leaves or the top leaves of infected squash plants were used, the third pellet was sometimes quite brown. Centrifugal analysis of suspensions of these brown pellets showed the color boundary to move with that of the major component. When such a preparation was passed through several extra cycles of differential centrifugation and resuspended in distilled water without addition of phosphate, the dark pigment usually separated from the major component, which retained its infectivity.

When noninfected control material of any age and from any of the plants employed was passed through the purification procedure, no visible pellet was obtained from the third high-speed centrifugation, even when 3 liters of noninfectious juice were concentrated into a single final tube. On the other hand, as little as 50 ml of juice from RSV-infected squash plants yielded a small but easily distinguishable pellet.

RSV CRYSTALS.—Concentrated suspensions of RSV (20 mg or more per ml) in 0.1 or 0.01 M phosphate buffer of pH 7 were quite milky in appearance. When these preparations were permitted to stand at 4°C for several hours, crystals developed and settled to the bottom of the container. Such crystals usually were plate-like with a diameter of 10 μ or less, which was 20–50 times their thickness (Fig. 2, A, B). Rosettes sometimes developed simultaneously with the disappearance of the plate-like crystals when microscope mounts were ringed with paraffin and allowed to stand at room temperature for 30 minutes. Gross crystals 0.05–1 mm or more in diameter developed in virus concentrates stored for 2–3 weeks in 0.1 M phosphate buffer of pH 7 (Fig. 2, C).

Plates similar to those shown (Fig. 2, A, B) also developed when concentrated virus suspensions in 0.01 N NaCl solution were stored at 4°C. This demonstrates that the phosphate ion is not essential to the formation of RSV crystals and that these crystals are not necessarily phosphoprotein complexes.

All forms of crystals were very fragile and disintegrated rapidly at room temperature or upon dilution of the suspension with water.

When plate-like crystals of RSV were washed in 0.1 M phosphate buffer of pH 7 and then dissolved in distilled water within 24 hours of crystal formation, the resulting suspension caused approximately the same number of lesions on test plants as did a non-crystallized suspension containing the same amount of protein. Most of the activity was lost, however, if the crystals were stored for a week or longer.

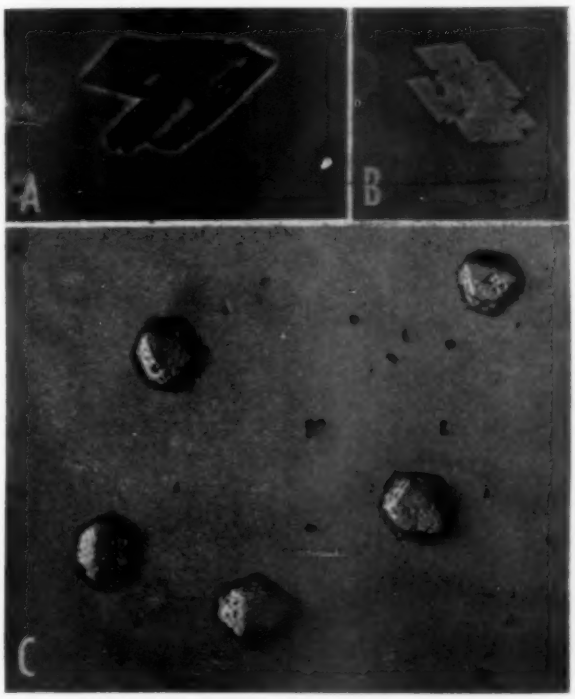

FIG. 2. Tobacco ringspot virus crystals. A, B) Plate-like crystals that developed during 12 hours' storage at 4°C of a concentrated virus suspension to which a few drops of 0.1 M phosphate buffer of pH 7 has been added. Magnification ×1500. C) Gross crystals that developed in a virus concentrate stored for 2 weeks in 0.1 M phosphate buffer of pH 7. Note that the crystals are decahedrons. ×150.

ELECTRON MICROSCOPY.—RSV purified by the procedure outlined was prepared in various ways for electron microscopy. Samples in distilled water, in 1 and 2 per cent formalin, in 2 per cent ammonium acetate, and in 2.5 per cent osmic acid were sprayed onto collodion-coated electron microscope grids and allowed to air dry. Other samples in distilled water were frozen dried following the procedure of Williams (18). All specimens were shadowed with uranium, and electron micrographs were taken with an RCA type EMU 2E electron microscope.

Electron micrographs of samples air-dried from distilled water suspension showed many collapsed particles and amorphous aggregates as well as some particles that appeared to be unaffected by desiccation (Fig. 3, A). On the other hand, only rigid noncollapsed particles that occurred singly or in small clusters were seen in electron micrographs of distilled water suspensions that had been diluted, prior to being sprayed, with 2 per cent ammonium acetate, with 1 or 2 per cent formalin, or with 2.5 per cent osmic acid (Fig. 3, B). When distilled water suspensions were frozen-dried, only distinct particles of uniform size and aggregates of such particles were observed (Fig. 3, C).

In micrographs of preparations fixed with 1 or 2 per cent formaldehyde, with 2.5 per cent osmic acid, or with 2 per cent ammonium acetate, many of the individual particles appeared to be somewhat angular (Fig. 3, B). When frozen-dried from distilled water, however, nearly all particles that appeared as individuals and many of those in the clusters were polyhedrons (Fig. 3, C, D). Many of the individuals were hexagonal in outline and cast shadows that were angular and provided some information regarding the third dimension of the particles. Although the particles were too small and the background too rough to permit determination of the precise shape of the particles from their outlines and those of their shadows, the shape that appears most likely to fit the observations is that of a rhombic dodecahedron.

The question arose as to whether or not the angular appearance was due to artefacts. That this was not the case is indicated by the fact that particles of 2 other viruses of almost identical size, turnip yellow mosaic virus and poliomyelitis virus, have been demonstrated in this laboratory (6, 13) to be spherical, or nearly so, when frozen-dried.

The total lengths of many rows of from 2 to 12 closely packed virus particles were measured. The measurements were compared with measurements of the diameters of Dow polystyrene latex (PSL) particles, lot 580 G, (3) that were present in the same micrographs and served as internal size-standards. The value used for the diameter of the PSL particles was 2590 A as reported by Backus and Williams (3).

An average value for the diameter of the RSV particles was determined for each of 15 micrographs representing 10 different purified preparations including some from each of the 4 hosts from which RSV was purified. These values ranged from 25.5 mμ to 26.6 mμ with a numerical average of 26 mμ. This value is believed to be more precise than that of 23 mμ reported by Desjardins, Senseney, and Hess (8), who calibrated their electron micrographs by using a microscope which had been calibrated with a replica of a grating with 15,000 lines to the inch.

Several of the best hexagons in frozen-dried prepa-

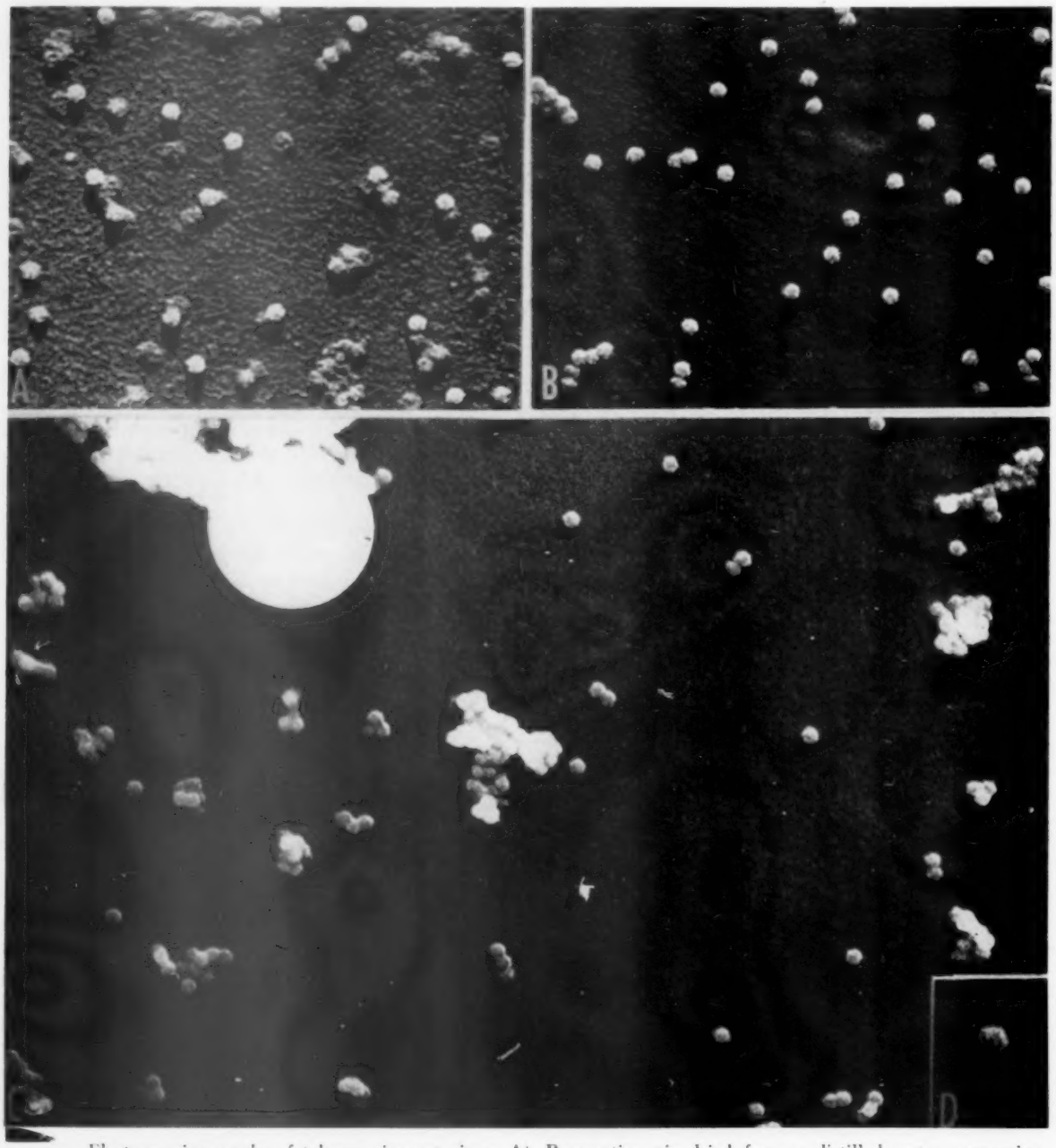

FIG. 3. Electron micrographs of tobacco ringspot virus. A) Preparation air dried from a distilled water suspension. Note that many particles appear collapsed, but that some appear to have retained their structural integrity. ×100,000. B) Air dried preparation like that of A but diluted with a 2 per cent formalin solution 2-3 minutes before the suspension was sprayed. Note some angularity of particles and the angular shadows. ×100,000. C, D) Preparation similar to that for A but frozen-dried from distilled water suspension. Note that the particles appear to be flat sided and that some are hexagonal in contour. Large white circle represents a particle of Dow polystyrene latex (PSL) which has a diameter of 2590 A. (C, ×100,000; D, ×200,000).

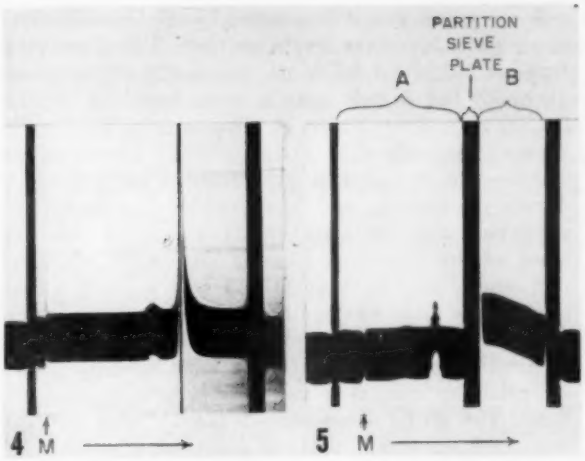

FIG. 4–5.—Fig. 4. Schlieren pattern showing position of boundaries of RSV preparation obtained from squash cotyledons after 28 minutes' centrifugation at 23,000 rpm. Suspension contained approximately 6 mg protein per ml in 0.01 M phosphate buffer of pH 7. Meniscus (M). Sedimentation constants are 116 S for the major component and 89 S for the minor component.—Fig. 5. Schlieren pattern showing position of boundaries in separation cell after 26 minutes' centrifugation at 20,410 rpm. Meniscus (M). Major boundary is obscured by the partition sieve plate. Minor component is to the left of partition in compartment A.

rations, including the one shown in Figure 3, D, were measured and found to have diameters of 24 mμ between opposite sides and 28 mμ between opposite points.

A sample of the strain of RSV used by Desjardins, Senseney, and Hess (8)[4] was also multiplied in squash cotyledons and in Turkish tobacco leaves. It, likewise, was found to have an average diameter of 26 mμ.

ULTRACENTRIFUGAL ANALYSIS.—Examination in the Spinco analytical ultracentrifuge of samples that appeared monodisperse in electron micrographs showed that these preparations usually contained 2 components; a major one with a sedimentation constant of 116 S and a minor one with a sedimentation constant of 89 S (Fig. 4).

One sample too small for analysis with the Spinco analytical ultracentrifuge was analyzed by the procedure described by Backus and Williams (5) in which a microcapsule was employed. The capsule was filled to the proper level with a virus suspension containing approximately 0.2 mg of protein per ml, and both ends were sealed in a flame (care was taken not to heat the contents). It was then "floated" in the cup of a Spinco SW 39 ("swinging cup") rotor and was centrifuged for 20 minutes at 20,000 rpm in a Spinco model L centrifuge. After this, the capsule was removed, washed, and inserted in a special micrometer adaptor (2) in a Beckman spectrophotometer. The capsule was moved in front of the slit of the spectrophotometer, and readings at 2 wave lengths (2600 A and 2800 A) were obtained for 45 positions throughout the length of the capsule. The virus inside the capsule was then resuspended and the suspension thoroughly mixed. It was then recentrifuged for 50 minutes at 20,000 rpm, after which readings at the same wave lengths were obtained for 47 positions. These 2 sets of data were plotted and smooth curves were drawn through the points (Fig. 6). From these curves it appears that this suspension was ultracentrifugally homogeneous with respect to particulate components with a sedimentation constant approximating that of the characteristic particles. The presence of less than 5 per cent of the slower moving component observed in Figure 4 might be indetectable, however, by this procedure. The importance of these data, therefore, is not that they demonstrate strict homogeneity of the sample but rather that the high ratio of absorption values at 2600 A and 2800 A throughout the length of each set show that the major component was a nucleoprotein of high nucleic acid content. Electron micrographs of this preparation revealed only the characteristic particles previously described, and the suspension proved to be highly infectious (capable of inducing 1–5 lesions per leaf when inoculated at a concentration of 0.005 μg per ml).

A separation cell (17) was used to ascertain whether or not the infectivity moved with the major or with the minor component. Both portions of the cell were filled with the virus suspension, and the cell was placed in the rotor and centrifuged at 23,000 rpm in a Spinco analytical ultracentrifuge until the boundary representing the major component had passed into the partition zone (Fig. 5). The rotor was then stopped and the suspension in compartment A was removed for comparison by local-lesion assay with samples of the starting suspension. From the position of the observable peak in compartment A, which showed the boundary position of the minor component, it was determined that 30 per cent of the slower moving component and a much smaller percentage of the major component remained in this compartment. The contents of this compartment were then removed and thoroughly mixed. If infectivity were associated di-

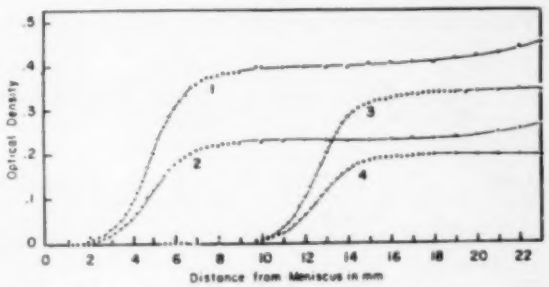

FIG. 6. Light absorption analysis of sedimentation boundaries of a RSV preparation (0.2 mg/ml) in 0.01 M phosphate at pH 7 centrifuged at 40,000 G in a field-aligning capsule. Curve 1) at 260 mμ, after 20 minutes; Curve 2) at 280 mμ after 20 minutes; Curve 3) at 260 mμ after 50 minutes; Curve 4) at 280 mμ after 50 minutes.

[4] The original material for this test was kindly supplied by Dr. R. P. Kahn, Camp Detrick, Maryland.

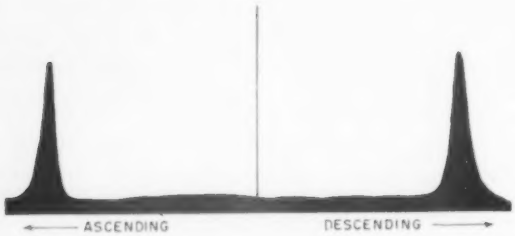

FIG. 7. Electrophoretic pattern of tobacco ringspot virus. Ascending and descending boundaries of a 15 mg/ml solution in 0.05 M phosphate buffer of pH 7 after 5 hours 16 minutes at a potential gradient of 2.5 volts/cm. Electrophoretic mobility was 11.3×10^{-5} cm/sec/volt/cm.

rectly with the slower-moving component, then this suspension from compartment A should have been as infectious as a suspension of the original preparation diluted to 30 per cent concentration. Such was not the case, for a 30 per cent suspension of the original preparation was 42 times as infectious as the contents of compartment A. As expected, the suspension from compartment B, which contained most of the major component and 70 per cent of the minor component, was found to be more infectious than the original preparation when compared at equal dilutions. It was obvious, therefore, that infectivity was associated with the faster-moving component rather than with the slower-moving one. Thus, these data provide evidence of association of infectivity with the characteristic particles that comprise the major component.

ELECTROPHORESIS.—A sample containing the 2 centrifugally resolvable components was examined by electrophoresis in a Spinco electrophoresis unit. Electrophoresis was conducted with a virus concentration of 15 mg/ml in 0.05 M phosphate buffer at pH 7, at a temperature of 1°C, and at a potential gradient of 2.65 volts/cm. Ascending and descending boundaries at the end of 5 hours 14 minutes are shown in Figure 7. Under the conditions employed, only 1 component was resolved during this period, and the electrophoretic mobility of this component was found to be 11.3×10^{-5} cm/sec/volt/cm.

PHOSPHOROUS ANALYSIS AND NUCLEIC ACID CONTENT.—Seven samples of RSV purified by the butanol-chloroform procedure were lyophilyzed, dried *in vacuo* over P_2O_5 at 105°C for 24 hours, and analyzed for phosphorous. The first 2 samples were found to contain less phosphorous (Table 1) than anticipated on the basis of Stanley's (15) earlier report. These pellets had been resuspended in glass distilled water at pH 6 during purification, and it was noted that under the conditions used the resuspension was rapid but that much of the virus was removed during the clarification procedure. Consequently it was suspected that some of the virus was being denatured and was losing its nucleic acid. Therefore, various salts were added to distilled water to change the ionic conditions and the pH in an attempt to avoid the suspected denaturation. The media employed (Table 1) were used to resuspend the first high-speed pellet; and, in some instances, the second one also. With the exception of samples 1, 2, 4, and 6, salt was removed from the virus suspensions by centrifugation. The virus was removed as a pellet, resuspended in glass-distilled water and pelleted again. These final pellets were lyophilyzed without being resuspended. Samples 1 and 2 had no salt added at any stage, whereas the virus of samples 4 and 6 was pelleted directly from the medium indicated. The ammonium acetate of sample 4 volatilized, whereas the retained salt in sample 6 probably accounts for the somewhat low phosphorous value. None of these salts appreciably affected the phosphorous content, and the mean value for phosphorous content was 3.13 per cent. On the assumption that RSV nucleic acid contains 9 per cent phosphorous, it was calculated that RSV contains 34.4 per cent nucleic acid. Although this represents a high nucleic acid content for a plant virus, it is somewhat below the 40 per cent estimated by Stanley (15).

YIELD OF RSV FROM SQUASH COTYLEDONS.—Nitrogen analysis of a purified RSV preparation that was concentrated from crude juice of infected squash cotyledons indicated a virus protein content equivalent to 45 mg per liter of starting material. An unrecorded amount of virus was later recovered from the clarification pellets but was not included in this figure.

INFECTIVITY.—As previously mentioned, suspensions of purified RSV were considered highly infectious if they induced the production of 1–5 lesions when

TABLE 1.—*Phosphorous analysis of various samples of tobacco ringspot virus*

Sample	Host	Suspending medium[a]	pH	Number of analyses	Phosphorous (percentage of dry weight)[b]
1	Squash	Glass-distilled H_2O	6.0	2	3.40
2	Squash	Glass-distilled H_2O	6.0	9	3.04
3	Squash	0.006 M sodium citrate	7.45	3	3.08
4	Turkish tobacco	Amonium acetate 0.5%	7.0	3	3.35
5	Squash	0.01 M $H_3BO_3 + Na_2SO_3$	7.0	3	3.12
6	Squash	0.01 M $H_3BO_3 + Na_2SO_3$	7.0	2	3.06
7	Squash	$H_2O + Na_2SO_3$	7.3	2	2.98

[a] Suspending medium listed is that in which the first (and in some instances the second) high-speed pellet of the sample was resuspended.
[b] Average of the analyses made for the sample.

rubbed onto leaves of black-eye cowpeas at a concentration of 0.005 μg per ml. In order to obtain further information concerning the infectivity of the characteristic particles of RSV, the spray-drop method of Backus and Williams (4) was used in an experiment designed to determine the number of particles present in the inoculum for each local lesion obtained. A suspension of purified RSV was diluted with a suspension of PSL particles of known concentration, and the mixture was sprayed onto electron microscope grids. The prepared specimens were then shadowed with uranium, and electron micrographs of 8 distinct drop residues were obtained. The number of RSV particles and the number of PSL particles that were present in the 8 residues were counted, and the concentration of RSV particles in the original suspension was calculated. A suspension containing approximately 6×10^9 particles per ml was then prepared and 0.5 ml of this was used as inoculum for 150 primary leaves of black-eye cowpea. An attempt was made to spread this inoculum over as much leaf surface as possible. The number of RSV particles in the inoculum used was approximately 3×10^9, and a total of 25,107 lesions was obtained. Thus 1 lesion was obtained for approximately every 120,000 characteristic particles in the inoculum.

STABILITY IN DIFFERENT SUSPENDING MEDIA.—The stability of RSV when stored in various suspending media at 4°C was examined (Table 2). It is evident that 1) the suspension stored for 83 days in 0.01 M phosphate buffer was more infectious than identical material stored at the same concentration in 0.1 M phosphate buffer, and 2) the suspension stored in 0.1 M phosphate buffer was more infectious than that stored in distilled water. These observations are in close agreement with the results obtained by Stanley (15). Local-lesion assays of recently prepared and stored suspensions of the same virus preparation suggest that dilute suspensions of virus stored at 4°C for 80 days in 0.01 M or 0.1 M phosphate buffers of pH 7 were more infectious than they were initially (Table 2). It is known, however, that many factors influence the production of local lesions. Different batches of test plants used on different days may vary greatly in susceptibility. On the other hand, it may be that most of the virus was present as aggregates in the distilled water suspensions and that it slowly became disaggregated upon storage in either phosphate buffer. If such is the case it is obvious that disaggregation did not occur immediately upon dilution since all samples were diluted with 0.01 M phosphate buffer for inoculation.

PURIFICATION OF OTHER PLANT VIRUSES BY THE BUTANOL-CHLOROFORM PROCEDURE.—The butanol-chloroform procedure employed in these studies was used also for the purification of tomato bushy stunt virus, southern bean mosaic virus, tobacco mosaic virus, and turnip yellow mosaic virus. It was found satisfactory as a procedure for obtaining essentially monodisperse suspensions of the characteristic particles of each of these viruses for electron microscopy. Suspensions of these viruses prepared by this procedure proved to be infectious, but adequate assays have not been made to determine how the infectivities of such suspensions compare with those of suspensions prepared in other ways. This procedure, however, produced pure suspensions of the infectious "bottom" component of turnip yellow mosaic virus from the juice of infected Chinese cabbage plants (6).

DISCUSSION.—Although only 1 infection was obtained for every 120,000 characteristic particles of RSV present in an inoculum, substantial evidence has been presented to show that infection is directly associated with these particles. The best preparations were found to contain 2 centrifugally distinguishable components, a major one representing 95 per cent or more of the total colloidal material and a minor one comprising the rest of the suspended particulates. One such preparation was centrifugally fractionated, and biological assays showed that infectivity was not associated with the minor component. Since only the 2 components were observed and the fraction which contained nearly all of the major component plus 70 per cent of the minor component was more infectious than the starting material, it was obvious that infectivity was

TABLE 2.—*Stability of tobacco ringspot virus*

Days in storage at 4°C	Dilution of stored concentrate	Suspending medium in which stored	Number of half leaves inoculated	Dilution at which inoculated	Average number of lesions per half-leaf[a]	Log-relative infectivity[b]
0			78	10^{-4}	12.2	5.0864
0			80	10^{-5}	1.18	5.0719
0			74	10^{-6}	0.1	5.0000
26	undiluted	H_2O	18	10^{-4}	2.5	4.3979
26	undiluted	H_2O	18	10^{-5}	None	
83[c]	10^{-3}	0.01 M PO_4 at pH 7	26	10^{-5}	17.5	6.2430
83[c]	10^{-3}	0.1 M PO_4 at pH 7	26	10^{-5}	5.65	5.7520
83[d]	10^{-3}	0.1 M PO_4 at pH 7	29	10^{-5}	6.3	5.7993
83[d]	10^{-3}	H_2O at pH 6.5	29	10^{-5}	1.07	5.0294

[a] Average number of local lesions that developed per half leaf on 40 inoculated half-leaves of black-eye cowpea following inoculation with indicated suspensions.
[b] Logarithm of the product of lesion count and total dilution factor.
[c] These preparations were compared on opposite half-leaves.
[d] These preparations were compared on opposite half-leaves.

associated with this material. Further evidence of the purity of the preparation and of the association of infectivity with the characteristic particles was obtained when crystals composed of these particles were washed, then dissolved, and found to possess most of the infectivity of the original suspension.

In preparations air dried from distilled water, some particles were collapsed whereas others appeared to retain their structural integrity. The question arises as to whether this is due to differences in the stability of different particles, to local differences in surface tension forces or ionic strength, or to some other factor prevalent during the final stages of drying from distilled water. If it could be shown that there is a real difference in the stability of different particles, then it would be important to know if this is related to infectivity. In any event, it is important to note that the suspending medium from which virus preparations are dried can have a profound effect on the appearance of the particles in electron micrographs.

Storage at 22°C in the presence of n-butanol and chloroform appears to be a necessary step in the present procedure for the purification of RSV. Several preliminary procedures were attempted for the purification of RSV, but all yielded virus concentrates of various degrees of impurity. Among these were the procedure used by Stanley (15) and several procedures employing the use of n-butanol and chloroform individually and in various combinations. All these procedures were conducted at 4°C. Several peaks were always observed when the final suspensions from each of these preparations were centrifugally analyzed. Electron micrographs of such preparations always revealed the presence of several components. Also, quantities of nucleoprotein similar to those obtained from infected plants were obtained from healthy ones.

The properties of RSV purified by the butanol-chloroform differential-centrifugation procedure agree very closely with those reported by Stanley (15). The former value reported for the sedimentation constant was 115 S. It is reported here as 116 S. Phosphorous analyses of recent samples lyophilyzed and dried over P_2O_5 gave 2.98 to 3.4 per cent phosphorous whereas the earlier report gave 3.2 to 4.1 per cent. This would suggest the possible loss of some phosphorous in the procedure described here or the presence of nonvirus phosphorous in Stanley's concentrates. The latter possibility is suggested by the inability to obtain homogeneous preparations by Stanley's procedure. The present studies also verify the fact that 0.01 M phosphate buffer is a better storage medium than either stronger phosphate buffers or distilled water.

Only in 1 property (particle diameter) was there a marked discrepancy between the present value and that calculated by Stanley (15). The earlier estimate of 19 mμ was calculated indirectly from ultracentrifugation, specific gravity, and ultrafiltration data, whereas the present value of 26 mμ was obtained directly from electron micrographs of uranium-shadowed drop residues containing particles of purified RSV and a few of polystyrene latex for internal calibration.

It is interesting to note the remarkable agreement between the values obtained for the present purified RSV samples and those obtained by Stanley in 1939. This agreement is all the more remarkable when one compares the degree of purity of the present samples with that of samples obtained by following Stanley's procedure. From the electrophoretic data presented by Desjardins, Senseney, and Hess (8) and from studies conducted in this laboratory, it would appear that Stanley's preparations probably had impurities amounting in some instances to as much as 40 per cent.

Tobacco ringspot virus is noted for its instability when stored at or near 22°C. The presence of n-butanol and chloroform in the clarified juice acts as a stabilizing agent, however, and prevents loss of infectivity. The butanol-chloroform procedure described here might prove very useful, therefore, for the purification of other unstable viruses that have been difficult to separate from contaminating components.

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THE IDENTITY OF BARN ROTS OF FLUE-CURED TOBACCO IN SOUTH CAROLINA [1]

Quintin L. Holdeman [2] and Walter H. Burkholder [3]

SUMMARY

When wet weather occurs during the tobacco growing and harvesting season in South Carolina, 2 distinct soft rots of ripe leaves may occur during early coloring stages of curing. One is caused by *Pythium aphanidermatum* and the other by a previously unidentified bacterium. The former organism produces a more or less uniform discoloration that spreads into the laminas as rapidly as through the veins of the leaves; the latter spreads first into the veins and later into the lamina, leaving islands of yellow tissue. On the basis of morphology, cultural characteristics, physiological characteristics, and cross inoculations, the bacterium was identified as *Erwinia aroideae* (Town.) Holland. Both pathogens were readily isolated, and successful infection experiments were conducted with each on mature tobacco leaves.

When wet, humid weather occurs during the growing and harvesting season in South Carolina, soft rots of ripe tobacco leaves may occur infrequently in the barn during the early coloring stages of curing. These rots are similar to the barn rot reported on flue-cured tobacco in Southern Rhodesia and attributed to *Rhizopus arrhizus* A. Fischer (6, 7). One of these rots has been briefly noted and reported as being caused by *Phythium aphanidermatum* (Edson) Fitzp. (3, 4). In 1951, a second barn rot associated with an unidentified bacterium was observed (5).

The purpose of this paper is to report on the identity of the bacterium, on pathogenicity tests with the 2 organisms, and on the relation of the bacterium to hollow-stalk in the field. Simple techniques for separating and isolating the 2 organisms are described.

THE DISEASE.—Barn rots begin as a watersoaked, brownish discoloration at the butt end of mature leaves that are tied to poles in the curing barn. As the decay progresses, the discoloration darkens to a brown or black and spreads rapidly through the leaf. At the end of the second or third day, a strong odor of decaying vegetable matter is noted. The slimy butt ends of the leaves begin to break, dropping the leaves to the floor of the barn. Once rot is well advanced, it is difficult to raise the heat in the barn sufficiently or rapidly enough to dry out the leaves in order to stop the advance of the decay. The amount of damage may vary from a few leaves to complete loss of the contents of the barn.

Conditions favoring the occurrence and spread of barn rots are harvesting during wet weather, placing wet tobacco in the barn, crowding the tobacco in the barn, and failing to provide sufficient ventilation to drive off excess moisture.

DIAGNOSES.—In the early stages of decay, the 2 barn rots were readily distinguished by the pattern of discoloration and rot produced. The irregular discoloration caused by the bacterium spread first into the veins and later into the lamina of the leaves, leaving islands of yellow tissue; in contrast, the more uniform discoloration produced by the fungus spreads into the lamina as rapidly as through the veins (Fig. 1).

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To confirm the diagnosis or to isolate the rot organisms, pieces of decayed material, collected before the barn temperatures were raised, were inserted into a slit about 2 in. above the severed base of vigorous tobacco suckers. The inoculated sucker was placed in an Erlenmeyer flask containing about ½ in. of water. To maintain high humidity in the flask, cotton was inserted around the stem of the sucker extending through the neck of the flask. If either of these 2 pathogens was present, about 24 hours following inoculation the stem of the sucker began to decompose around the point of inoculation, and in 48–52 hours, a considerable portion of the stem showed soft rot. When the fungus was present, a conspicuous white tuft of mycelium appeared on the rotten area. Discoloration did not extend into the leaf veins. When the bacterium was present, the soft rot and discoloration extended up into the veins of the leaves on the sucker, and discolored leaf veins contained an abundance of motile bacteria.

Isolation of pathogens.—When isolations were attempted directly from decayed leaves, difficulties were encountered because of the abundance of other fungi and bacteria. Greater efficiency was facilitated by inserting decayed tissue into tobacco suckers as described, followed by isolation from the discolored areas produced.

Tufts of fungus mycelium transferred from the tobacco sucker to water agar fruited in 18–48 hours. Both sporangia and oospores were produced on water agar. From studies of several isolates from barn rot, from stem rot of tobacco transplants (3), and from a stalk rot of large tobacco plants that occurs during wet weather, it was concluded that the morphology of the fungus fits within the description of *Pythium aphanidermatum* reproduced by Middleton (8) in his monograph of the genus.

To isolate the bacterium, small pieces were cut from the discolored leaf vein of the tobacco sucker. Each piece of tissue was washed through several changes of sterile water and triturated in a sterile water blank. The resulting suspension was diluted through a series of water blanks, and these were poured over petri dishes containing hardened potato-dextrose agar. Transfers were made from the few isolated colonies that developed on the dishes receiving the greatest dilutions.

Pathogenicity.—For pathogenicity tests, mature tobacco leaves were harvested from the field. Each isolate of the bacterium or of the fungus was injected into a leaf midrib from the lower side near the butt of the leaf. Control leaves were punctured but did not receive inoculum. Each leaf was placed separately

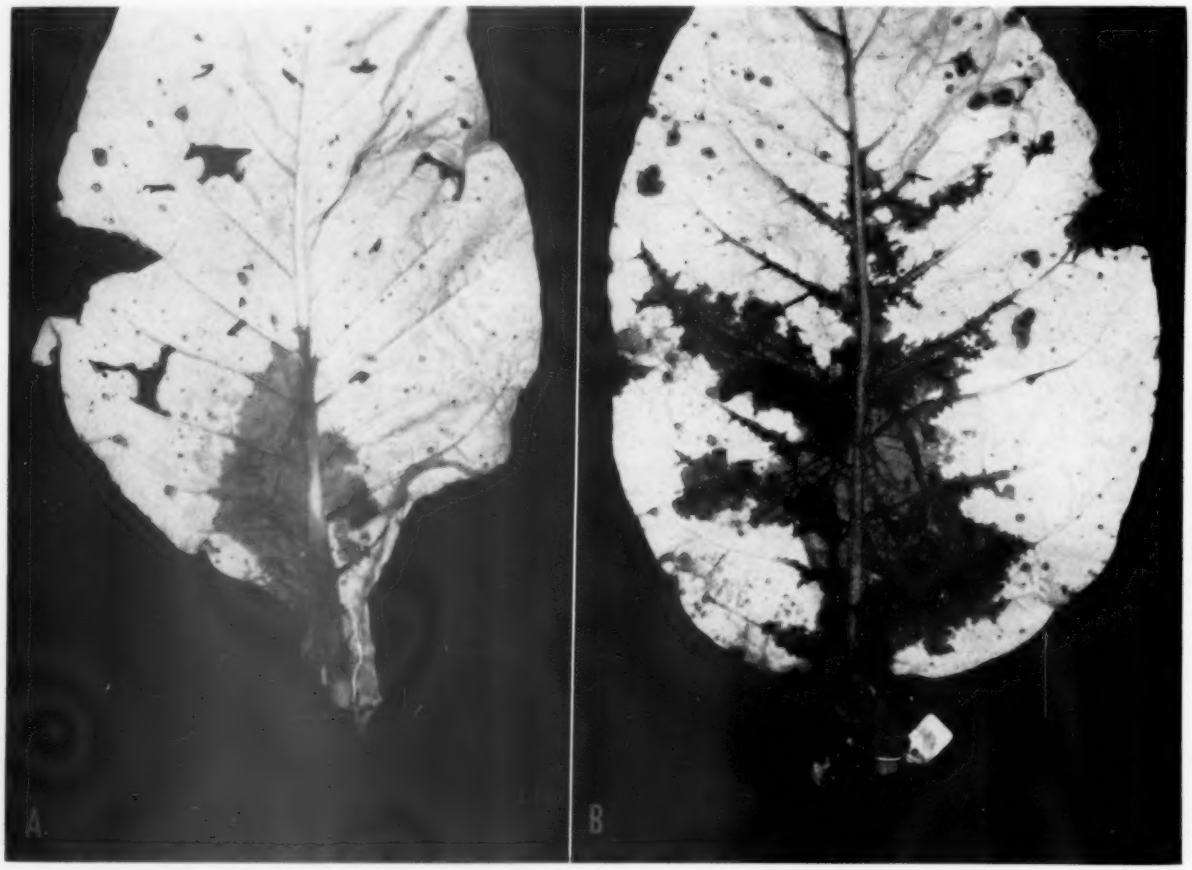

Fig. 1. Early stages of barn rot following inoculation by A) *Pythium aphanidermatum* and B) *Erwinia aroideae*.

between wet toweling or newspaper. The leaves were then bulked together, wrapped with wet cotton cloth, and incubated at 25–28°C. Typical decay symptoms (Fig. 1) occurred in 24–48 hours. No decay was evident in the controls. Repeated tests yielded the same results.

Insertion of bits of the inoculated leaves into tobacco suckers yielded the same results described previously for the 2 organisms, i.e. the fungus grew out of the stem of the decaying sucker or motile bacteria were present in the discolored leaf veins of decayed suckers.

Bacterial barn rot was associated with the occurrence of "hollowstalk", a soft rot of the pith of plants in the field. Decayed material obtained from diseased field plants, from decaying leaves out of a curing barn, and from diseased transplanted seedlings affected with a bacterial soft rot produced the same reaction when inserted into detached suckers or into mature tobacco leaves.

Pythium barn rot was reproduced by inoculation of mature tobacco leaves with isolates of *P. aphanidermatum* from barn rot, from stem rot of tobacco transplants, and from a stalk rot of large tobacco plants. All isolates were capable of causing stem rot of transplants when cool wet weather occurred during transplanting season. Isolates of *P. ultimum* Trow from stem rot of transplants did not produce barn rot following inoculation. When *P. ultimum* was inserted into tobacco suckers, as described above, it produced a decay of the pith but failed to produce a rapid decay of the outer layers of the stem.

THE BACTERIAL PATHOGEN.—The following description of the bacterium causing the barn rot of tobacco is based on 3 isolates. One was isolated from decayed leaves received from near Sumter, South Carolina, and the other 2 were isolated from decayed leaves obtained near Darlington, South Carolina. The pure culture methods used in this study are the same as those employed by Burkholder and Smith (2) in describing *Erwinia atroseptica* (v. Hall) Jennison and *E. carotovora* (L. R. Jones) Holland.

Morphology.—The bacteria from 24-hour cultures on beef-peptone agar are straight rods, the majority of which are 1.5–3μ in length, although a few short rods occur. They are gram-negative and motile with 1–6 peritrichous flagella.

Cultural characteristics.—On beef-peptone agar slants at 27°C, growth is filiform, white to cream in color. In 24 hours, growth is light, but in 48 hours it is moderate to good. Broth is turbid in 24 hours, but no pellicle is formed. In litmus milk, litmus is reduced, a curd is formed in 3 days, and ¼ of the medium is whey in 7 days. On Endo agar slants, growth appears in 24 hours and becomes a deep red in color, as does the medium. There is good growth on Krumwiede's triple sugar agar and the medium turns yellow, but in 2 weeks a red coloration appears at the top of the agar. A white growth appears in Clara's solution, which becomes very turbid. In Fermi's solution, growth appears in 24 hours and becomes flocculent and white. In Uschinsky's solution, a white growth appears, the medium becomes turbid, and a heavy sediment forms. This pathogen causes much greater turbidity in these 2 solutions than does *E. atroseptica* or *E. carotovora*. Good growth that is a definite pink in color occurs in 24 hours on desoxycholate agar, but in 1 week yellowish colonies appear over the early growth. In milk containing 0.05 per cent methylene blue, the lower half of the tube is white in 24 hours, but the blue color returns in 1 week.

Physiological characteristics.—Growth in gelatin stabs appears in 24 hours, liquefaction begins in 2 days, and the gelatin is half liquefied in 1 month. Indol is not formed in tryptone broth, but in 7–10 days H_2S production causes a definite blackening of lead acetate paper suspended above this medium. Nitrates are rapidly reduced to nitrites. The methyl red test is weakly positive, whereas the Voges-Proskauer test is strongly positive. Lipase is negative with the Starr method. There is good growth in broth plus 5 per cent NaCl, and a delayed but good growth in broth plus 7 per cent NaCl. Growth occurs at 6°C after 10 days, it is good at 37° in 24 hours, and fair at 40°. No growth occurs at 42–43°. In shake cultures using dextrose nutrient agar, the pathogen grows readily 2 cm or more below the surface, showing that it is a facultative anaerobe.

Acid but no gas is produced in a synthetic medium plus dextrose, galactose, levulose, arabinose, xylose, lactose, sucrose, glycerol, mannitol, or salicin. Growth in maltose medium is doubtful. An alkaline reaction occurs in sodium citrate medium, but there is only slight growth in sodium tartrate medium even after 10 days. There is no growth in medium containing dulcitol, ethyl alcohol, sodium hippurate, or sodium malonate. Starch is not hydrolysed. Ammonium pectate medium is liquefied.

The pathogen utilizes peptone, gelatine, asparagine, ammonium salts or nitrates as nitrogen sources.

Cross inoculations.—Since the tobacco isolates in culture appear to have all the generic characteristics of the soft rot bacteria, tests were made to determine whether they could infect and rot the vegetables usually attacked by these pathogens. Inoculations, therefore, were made on a number of the usual suscepts, and readings were taken 24 hours later. The following were shown to be very susceptible: potato tubers, carrots, cauliflower, lettuce, onion bulbs, celery, and ripe tomato fruits. Inoculations on stems of *Sedum spectabile* resulted in no infection, although concomitant inoculations with *Erwinia chrysanthemi* Burk., McFadden, et Dimock (1) caused considerable and rapid stem decay. Light infection was obtained on young calla plants and corms.

Taxonomy.—The cultural characteristics of the pathogen and its ability to rot vegetables definitely places it in the genus *Erwinia*. The tobacco pathogen, however, differs from *E. carotovora* in its inability to

utilize ethyl alcohol, dulcitol, and the sodium salts of hippuric, malonic, and tartaric acids. It is similar to *E. atroseptica* in this respect, but it differs from this species and also from *E. carotovora* in its action on Krumwiede's triple sugar agar, its very turbid growth in Fermi's and Uschinsky's solution, its Voges-Proskauer test, its ability to grow in 7 per cent NaCl media, its good production of H_2S, and its ability to grow at 40°. It differs from *E. chrysanthemi* in, among other things, its carbohydrate utilization and its inability to decolorize bromthymol blue. It is a facultative anaerobe, whereas the other 3 soft rot species of *Erwinia* are strict aerobes.

The facts that the tobacco bacterium appears somewhat longer in size than the other 3 soft rot Erwinia, that it produces H_2S readily, that it has a very high maximum temperature for a plant pathogen (40°C), that it produces an extremely high turbidity and sediment in Uschinsky's solution, and that it does not produce gas in a sugar medium place it within the description of *Erwinia aroideae* as reported originally by Townsend (9). The absence of gas production is not a limiting character for this species of *Erwinia* since many isolates of the microaerogenic *E. atroseptica* and *E. carotovora* do not produce a demonstrable amount of gas; nevertheless, its absence in this pathogen agrees with Townsend's description. Of over 100 soft rot bacterial cultures of the genus *Erwinia* studied by the junior author, these 3 isolates are the only ones that might be considered *E. aroideae*. It is very difficult to determine with confidence which soft rot bacterium is being referred to as *E. aroideae* in the literature, since such reports are based entirely on the absence of gas production. The failure to form gas is certainly not a dependable character.

The only character that might throw some doubt on its position here is that the bacterium is weakly virulent to calla lilies. In other species of pathogens, however, there are strains showing various degrees of virulence, hence this character is probably not a valid reason for placing the pathogen elsewhere. Furthermore, Wolf (10) has reviewed the literature on the hollow-stalk disease of tobacco and considers the causal organism to be *E. aroideae*. Therefore, on the basis of the various reasons stated above, the causal pathogen of the bacterial barn rot of tobacco is held to be *Erwinia aroideae* (Town.) Holland.

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BEETLE TRANSMISSION, HOST RANGE, AND PROPERTIES OF SQUASH MOSAIC VIRUS [1]

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SUMMARY

Squash mosaic virus in juice of infected Zucchini squash plants had a dilution tolerance of 1 to 1,000,000 and a thermal inactivation point of 75°C. The virus was still active in juice aged for 6 weeks *in vitro* at room temperature and in juice that had been kept frozen for 5 years.

Eleven species of Cucurbitaceae, 2 species each of Leguminosae and Umbelliferae, and 1 species of Hydrophyllaceae were found to be susceptible to the virus. Except for those species belonging to the Cucurbitaceae, infected plants showed no distinct symptoms. No symptoms developed on a total of 88 other species of plants in 29 families inoculated with the virus, nor was the virus recovered from any of them. None of the 12 species of Solanaceae tested, including species of tobacco commonly utilized as test plants in virus research, were found to be susceptible.

The western striped cucumber beetle, *Acalymma trivittata* (Mann.), and the western twelve spotted cucumber beetle, *Diabrotica undecimpunctata undecimpunctata* Mann., were both found to transmit the squash mosaic virus, but they both failed to transmit cucumber mosaic virus to squash plants.

When transferred daily to healthy squash plants, infective western striped cucumber beetles retained the squash mosaic virus for 17 days, and the western spotted cucumber beetle retained it for 20 days. An individual striped cucumber beetle infected 9 of 28 squash plants when transferred daily to a healthy plant. The western striped cucumber beetle acquired the virus after only 5 minutes' feeding on a diseased leaf following 72 hours of fasting.

Fluid regurgitated by the beetles was found to be highly infectious. This suggests regurgitation as the most likely source of virus in transmission. Feces from beetles was shown to contain active virus. The virus was recovered from the blood of beetles previously fed on virus-infected plants as well as from the crushed bodies of such beetles.

Nine species of aphids tested as vectors of squash mosaic virus were found incapable of transmitting the virus. Five species of leafhoppers and 2 species of plant bugs also were found not to be vectors of the virus.

Squash mosaic occurs most commonly in the coastal regions of California, but it also has been found in the interior valleys. The disease is characterized by severely mottled and malformed foliage with raised dark green blister-like areas (15). Preliminary reports (6, 7) indicated the virus to be highly infectious, to be transmitted by 2 species of cucumber beetles, and to have a limited host range. Kendrick (11) and Middleton (15) have reported the virus to be seed borne in muskmelon and squash.

Electron micrographs of squash mosaic virus by Takahashi and Rawlins (20) indicate that the virus particles are spherical with an average diameter of 30 mμ. The virus has been crystallized by Takahashi (19).

Squash mosaic virus is of particular interest in that it is one of a small group of viruses that are transmitted by insects with mandibulate or chewing mouthparts. Several plant viruses have been transmitted by mandibulate insects: cowpea mosaic virus by the bean leaf beetle (2, 3, 16); potato spindle tuber virus by grasshoppers, flea beetles, and larvae of the Colorado potato beetle (9); yellow turnip mosaic virus by flea beetles (14, 17), mustard beetles, grasshoppers, and earwigs (14); tobacco mosaic virus by grasshoppers (21) and caterpillars (18); and potato latent mosaic and tobacco ringspot viruses by grasshoppers (21).

Markham and Smith (14) have suggested that of the mandibulate insects, only those having the habit of regurgitation are capable of acting as vectors of plant viruses. Feces, regurgitated juice, and the crushed bodies of beetles have been demonstrated to contain active virus (3, 8). Viruses transmitted by mandibulate insects are highly infectious, and some are retained by their insect vectors for as long as 14 days (3). These viruses apparently are transmitted only by mandibulate insects and not by any suctorial insects such as aphids (3, 13, 14, 17) except possibly potato spindle tuber virus (9), which was reported to be transmitted also by tarnished plant bugs. Cabbage mosaic (12) and cucumber mosaic (4) viruses, however, are typical nonpersistent aphid-borne viruses that have been reported also to be transmitted by mandibulate species, but these reports need to be substantiated.

The study of squash mosaic virus was undertaken to determine the symptoms, physical properties, host range, and insect vector relationships as an aid to identification and differentiation of the virus from other viruses infecting cucurbits. Various insect species were tested to establish how the virus was spread in the field. The relationships of the virus to cucumber beetles was investigated to determine the acquisition, transmission, and retention of the virus by the vector. Feces, regurgitated juice, and crushed bodies of beetles were tested for active virus in an effort to establish the method of transmission.

SYMPTOMS.—Middleton (15) briefly described the

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most conspicuous and characteristic symptoms of squash mosaic as consisting of filiform leaves resulting from a reduction of interveinal tissue. The leaves may be distorted and mottled and show dark-green raised areas. The fruits from infected vines are malformed with raised domelike swellings.

Symptoms not previously described are presented here. The first symptoms on young seedlings of squash and pumpkin develop about 7 days after inoculation and consist of vein clearing and chlorotic spotting of the younger leaves. These leaves tend to cup upward and to develop a mottling of light and dark green areas. The most characteristic symptoms on experimentally inoculated squash plants are regular marginal projections of the veins (Fig. 1, A) resulting from retarded development of interveinal tissue, followed by a severe distortion of leaves as previously described (15) (Fig. 1, B). In advanced stages of the disease, leafy outgrowths or enations develop from the lower leaf surface of some varieties of squash and pumpkin. Conspicuous enations were noted particularly on naturally infected African Belle squash (Fig. 1, C). Infected squash are stunted and somewhat chlorotic.

Cucumbers infected with squash mosaic virus develop

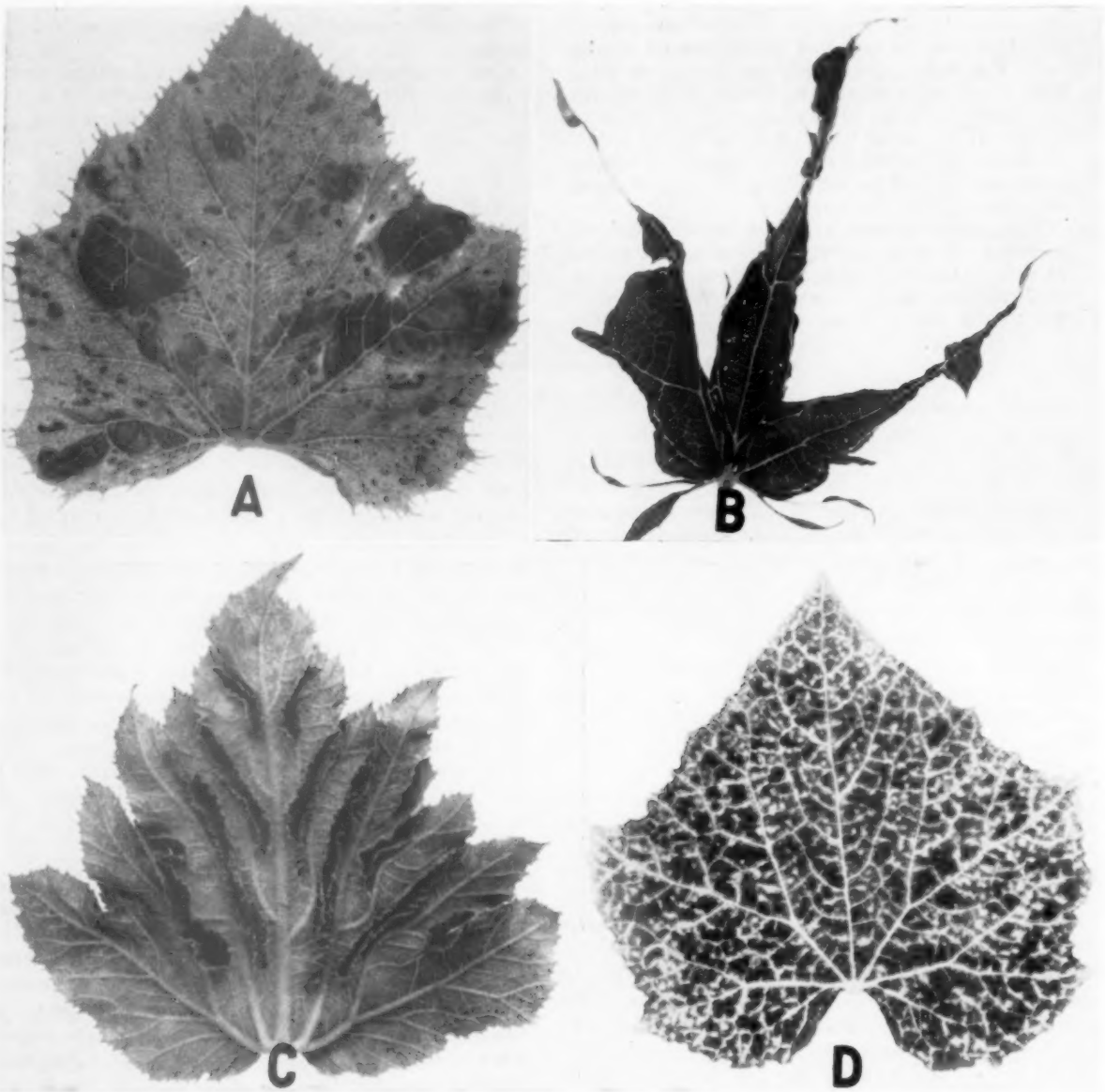

FIG. 1. Symptoms in leaves of plants experimentally inoculated with squash mosaic virus. A) Leaf of Early White Bush Scallop squash showing mottling, dark green blister-like raised areas, and characteristic marginal projection of veins. B) Severely malformed Early White Bush Scallop squash leaf resulting from reduced interveinal tissue. C) Leaf of naturally infected African Bell squash, showing enations. D) Cucumber leaf with prominent vein clearing, yellow vein banding, and spotting.

numerous circular chlorotic spots on the inoculated cotyledons. The first true leaves also show yellow spotting, followed by a prominent vein clearing (Fig. 1, D) and yellow vein banding. The young leaves are often curled and cupped upward. The subsequent leaves develop few or no symptoms, and symptoms on the first true leaves tend to fade out so that it is difficult to diagnose the disease on cucumbers several weeks following the onset of infection. This probably explains why the disease has not been observed to occur naturally on cucumbers even in areas where it is endemic on squash.

Muskmelons inoculated with squash mosaic virus develop only a slight vein clearing, followed by mottling, yellow spotting, and green vein banding. Other leaves may show yellow streaking, yellow spotting, or general yellowing along the veins. A few plants develop slightly malformed leaves on which the veins protrude beyond the leaf margin. Cantaloupes and Crenshaw, Casaba and Honey Dew melons are only mildly affected by the virus.

No symptoms were observed on experimentally inoculated plants of *Phacelia campanularia* Gray, sweet pea, garden pea, West India gherkin, coriander, or salad chervil, although virus was recovered from these plants.

PHYSICAL PROPERTIES.—Zucchini squash (*Cucurbita pepo* L. var. *melopepo* Alef.) was utilized both as source plant and test plant. Each test consisted of 5 or 6 replicates, each of 5 plants. The test plants were used about 7 days after the seed had been planted and while the plants were still in the cotyledon stage, before any true leaves had developed. Juice was extracted from virus source plants about 2–4 weeks after they first showed symptoms. The leaves of test plants were dusted with Carborundum powder before they were inoculated by rubbing with a cotton swab moistened with infective juice. The plants were washed with water a few minutes after they had been inoculated. The thermal inactivation and aging studies were conducted by adding 5 ml of undiluted infective squash juice to each test tube, which was then plugged with cotton. In the thermal inactivation tests, the test tubes were immersed in a hot water bath equipped with a mechanical stirrer. The temperature was controlled to within ¼°. At the end of 10 minutes the test tube was immediately plunged into ice water in order to cool the juice quickly.

Squash mosaic virus occurs in high concentration in infected Zucchini squash plants (Table 1). One of 30 plants inoculated with juice diluted 1 to 1,000,000 with distilled water became infected. Dilutions of 1 to 10,000 were infectious to 21 of 30 test plants.

Thermal inactivation occurred between 70 and 75°C during a 10-minute exposure. The number of infections resulting from inoculation of 30 Zucchini plants with infectious juice heated for 10 minutes at the indicated temperatures were as follows: 22°, 30; 60°, 26; 65°, 30; 70°, 22; 75°, 0; 80°, 0; and 85°, 0.

The virus is resistant to aging. Juice of infected squash stored at room temperature was often infective after 6 weeks, but not after 2 months. The numbers of infections following the inoculation of 25 Zucchini plants with infectious juice stored at room temperature for the indicated periods were as follows: 0, 23; 1 week, 24; 2 weeks, 25; 3 weeks, 23; 4 weeks, 19; 5 weeks, 16; and 6 weeks, 6. In another test, the number of infections were as follows: 0, 25; 1 month, 23; 2, 3, 4, 5 and 6 months, 0.

TABLE 1.—*Number of infections following inoculations of Zucchini squash plants with various dilutions of juice from Zucchini plants infected by squash mosaic virus*

Dilution	Number of plants infected[a]
Undiluted	30/30
1:10	30/30
1:100	28/30
1:1,000	29/30
1:5,000	23/30
1:10,000	21/30
1:100,000	4/30
1:200,000	5/30
1:300,000	3/30
1:500,000	0/30
1:600,000	2/30
1:700,000	2/30
1:800,000	0/30
1:1,000,000	1/30

[a] Numerator is number of plants infected, and denominator is number inoculated.

Squash mosaic virus was demonstrated to be resistant to inactivation by freezing. The virus was still active after 5 years in frozen juice.

HOST RANGE.—Approximately 100 species of plants in 33 families were tested for their susceptibility to the squash mosaic virus. At least 5 young seedlings of each species were inoculated. Mechanical inoculations were made by dusting with Carborundum and rubbing the leaves with cotton swabs moistened with juice from infected Zucchini squash plants. With many species, a second set of 5 plants were inoculated, and in a few cases a third or even a fourth set of 5 plants were tested. The species of Cucurbitaceae usually were inoculated during the cotyledon stage, whereas plants belonging to most other families were inoculated during the 2- to 4-leaf stage. Attempts to recover the virus were made usually about 1 month following inoculation of the plants. If symptoms developed only on some of the inoculated plants, recovery tests were made with juice extracted from a few leaves of such plants. If no symptoms developed, several leaves were taken from each of the 5 inoculated plants and the extracted juice used to inoculate 5 Zucchini squash plants.

Of the 12 species belonging to the Cucurbitaceae that were tested, 11 were found readily susceptible to the squash mosaic virus, and only watermelon, *Citrullus vulgaris* Schrad., failed to develop infection. Two varieties of *C. vulgaris* Schrad. var. *citroides* Bailey, large green and red seeded citron, became infected readily and developed severe symptoms. Five species

of plants belonging to the families Hydrophyllaceae, Leguminosae, and Umbelliferae were found to be susceptible. Although none of these 5 species developed any reliable symptoms, virus was recovered from them.

Plants found susceptible to squash mosaic belong to the following varieties and species: CUCURBITACEAE—Field pumpkin, *Cucurbita pepo* L., var. Connecticut or Large Yellow, and Table Queen; bush squash, *Cucurbita pepo* L. var. *melopepo* Alef., var. Early White Bush Scallop, Early Yellow Summer Crookneck, and Zucchini; Kentucky field pumpkin, *Cucurbita moschata* Duch.; autumn and winter squashes, *Cucurbita maxima* Duch., var. Hubbard, Delicious, and Banana; striped cushaw pumpkin, *Cucurbita mixta* Pang.; white flowered gourd, *Lagenaria siceraria* Standl., var. Bottle and Dipper; citron or preserving melon, *Citrullus vulgaris* Schrad. var. *citroides* Bailey, var. Large Green and Red Seeded; West India or bur gherkin, *Cucumis anguria* L.; cucumber, *Cucumis sativus* L., var. Klondyke, Japanese Climbing, Lemon, Boston Pickling, Crystal Apple, Straight-8, Snow's Pickling, Early Cluster, Improved Long Green, Davis Perfect, and Early White Spine; muskmelon, *Cucumis melo* L., var. Hearts of Gold, Golden Honey Dew, Honey Rock, Emerald Gem, Honey Dew, No. 45 Powdery Mildew Resistant, Crenshaw, Casaba, and Persian; serpent or snake gourd, *Trichosanthes anguina* L.; and balsampear, *Momordica charantia* L. HYDROPHYLLACEAE—*Phacelia campanularia* Gray. LEGUMINOSAE—Sweet pea, *Lathyrus odoratus* L., var. Spencer; and garden pea, *Pisum sativum* L., var. Perfection and Laxton's Progress. UMBELLIFERAE—Coriander, *Coriandrum sativum* L.; and salad chervil, *Anthriscus cerefolium* Hoffm.

Although sweet pea and garden pea were found susceptible, 19 other species listed below were nonsusceptible. Coriander and salad chervil were experimentally infected, but 6 other species of Umbelliferae were immune.

Mechanical inoculation with squash mosaic virus resulted in no infection of any of the following 88 species of plants representing 29 families: AMARANTHACEAE—Feathered celosia, *Celosia argentea* L.; and rough pigweed, *Amaranthus retroflexus* L. APOCYNACEAE—Madagascar periwinkle, *Vinca rosea* L. ASCLEPIADACEAE — Butterfly-weed, *Asclepias tuberosa* L. BORAGINACEAE — Chinese forget-me-not, *Cynoglossum amabile* Staft & Drummond. CAMPANULACEAE—Tussock bellflower, *Campanula carpatica* Jacq.; and Canterbury bells, *Campanula medium* L. CARYOPHYLLACEAE—Carnation, *Dianthus carophyllus* L. CHENOPODIACEAE—Sugar beet, *Beta vulgaris* L.; spinach, *Spinacia oleraceae* L., var. Virginia Savoy and Long Standing Bloomsdale; summer cypress, *Kochia scoparia* Schrad.; fogweed, *Atriplex expansa* (D. & H.) Wats.; and Bractscale, *Atriplex bractoesa* Wats. COMPOSITAE—Sunflower, *Helianthus annuus* L.; zinnia, *Zinnia elegans* L., var. Golden Gem and Scarlet Gem; cos lettuce, *lactuca sativa* L. var. *longifolia* Lam.; lettuce, *Lactuca sativa* L., var. New York; cosmos, *Cosmos bipinnatus* Cav.; pot marigold, *Calendula officinalis* L.; French marigold, *Tagetes patula* L.; African marigold, *Tagetes erecta* L.; Cape marigold, *Dimorphotheca sinuata* DC; China aster, *Calistephus chinensis* Nees; and feverfew, *Chrysanthemum parthenium* Pers. CONVOLVULACEAE—Imperial Japanese morning glory, *Impomoea nil* Roth. CRUCIFERAE—Annual stock, *Matthiola incana* R. Br. var. *annua* Voss; cabbage, *Brassica oleracea* L. var. *capitata* L.; cauliflower, *Brassica oleraceae* L. var. *botrytis* L.; dames violet, *Hesperis matronalis* L.; globe candytuft, *Iberis umbellata* L.; golden-tuft, *Alysum saxatile* L.; radish, *Raphanus sativus* L., var. White Icicle; turnip, *Brassica rapa* L. var., Purple Top White Globe; wallflower, *Cheiranthus cheiri* L.; and watercress, *Nasturtium officinale* R. Br. CUCURBITACEAE — Watermelon, *Citrullus vulgaris* Schrad., var. Monte Cristo, Dixie Queen, Winter King and Queen, Black-seeded Angleno, Stone Mountain, Florida Favorite, Klondyke, and Tom Watson. EUPHORBIACEAE — Snow-on-the-mountain, *Euphorbia marginata* Pursh.; and castor bean, *Ricinus communis* L. GERANIACEAE—Zonal geranium, *Pelargonium zonale* Ait. GRAMINEAE—Sweet corn, *Zea mays* L., var. Alameda. HYDROPHYLLACEAE—Baby-blue-eyes, *Nemophila menziessii* Hook & Arn. LEGUMINOSAE—Alfalfa, *Medicago sativa* L., var. California Common; bur clover, *Medicago hispida* Gaertn.; blackeye cowpea, *Vigna sinensis* Savi; broad bean, *Vicia faba* L.; kidney bean, *Phaseolus vulgaris* L., var. Bountiful; lima bean, *Phaseolus linensis* Macf. var. *limenanus* Bailey; soy bean, *Glycine max* Merr.; yard long bean, *Vigna sesquipedalis* Fruwirth; garden pea, *Pisum sativum* L., var. Alaska, Alderman, and Little Marvel; crimson clover, *Trifolium incarnatum* L.; white clover, *Trifolium repens* L.; Ladino clover, *Trifolium repens* L. var. *latum;* red clover, *Trifolium pratense* L.; white sweet clover, *Melilotus alba* Desr.; sweet clover, *Melilotus indica* All.; yellow sweet clover, *Melilotus officinalis* L.; peanut, *Arachis hypogaea* L.; *Lupinus hartwegii* Lindl.; and *Lupinus polyphyllus* Lindl. MALVACEAE—Cotton, *Gossypium hirsutum* L., var. Alcala; okra, *Hibiscus esculentus* L. NYCTAGINACEAE—Four-o'clock, *Mirabilis jalapa* L. PAPAVERACEAE—California poppy, *Eschscholtzia californica* Cham. POLEMONIACEAE—Annual phlox, *Phlox Drummondii* Hook. POLYGONACEAE—Garden sorrel, *Rumex acetosa* L. PLUMBAGINACEAE—Sea lavender, *Limonium sinuatum* Mill. PRIMULACEAE—Cowslip, *Primula veris* L. RANNUNCULACEAE—Columbine, *Aquilegia* sp.; and larkspur, *Delphinium* sp. annual. RESEDACEAE—Common Mignonette, *Reseda odorata* L. SCROPHULARIACEAE—Snapdragon, *Antirrhinum majus* L.; and common foxglove, *Digitalis purpurea* L. SOLANACEAE — Pepper, *Capsicum frutescens* L. var. *grossum* Bailey; jimson weed, *Datura stramonium* L.; common garden petunia, *Petunia hybrida* Vilm. var. Rosy Morn; potato, *Solanum tuberosum* L.; tomato, *Lycopersicum esculentum* Mill., var. Marglobe; tobacco, *Nicotiana tabacum* L., var. Turkish, Havana, White Burley, and Connecticut Seed Leaf; tree tobacco, *Nicotiana glauca* Graham;

Nicotiana glutinosa; Nicotiana alata Link & Otto var. *grandiflora* Comes; *Nicotiana langsdorfi; Nicotiana rustica* var. *hummulus;* and *Salpiglossis sinuata* Rinz & Pav. TROPAEOLACEAE—Garden nasturtium, *Tropaeolum majus* L. UMBELLIFERAE—Celery, *Apium graveolens* L. var. *dulce* Pers.; blue lace-flower, *Trachymene caerulea* R. Graham; carrot, *Daucus carota* L. var. *sativa* DC; parsnip, *Pastinaca sativa* L.; parsley, *Petroselinum crispum* Nym.; and caraway, *Carum carvi* L.

None of 12 species of Solanaceae, including 6 species of tobacco commonly utilized as test plants in virus experiments, were found to be susceptible. These results provide a means of differentiating squash mosaic virus from cucumber mosaic virus. The host range of squash mosaic virus is relatively limited when compared to that of cucumber mosaic. Squash mosaic was not transmitted to any of the 5 species of Chenopodiaceae or any of the 10 species of Cruciferae and Compositae tested.

ATTEMPTS TO TRANSMIT SQUASH MOSAIC VIRUS BY MEANS OF APHIDS, LEAFHOPPERS, AND PLANT BUGS.—Nine species of aphids were tested as possible vectors of squash mosaic virus. Four of the 9 species occur naturally on squash in the field in California. These are the cotton or melon aphid, *Aphis gossypii* Glover; the green peach aphid *Myzus persicae* (Sulz.); the potato aphid, *Macrosiphum solanifolii* (Ashm.); and the pea aphid, *Acyrthosiphon onobrachis* (BdF.) (*M. pisi* (Kalt.)). The other 5 species were the celery aphid, *Aphis apii* Theo.; the rusty banded aphid, *Yezabura inculta* (Walker) (*Aphis ferruginea-striata* Essig); the dock aphid, *A. rumicis Linn.;* the crescent-marked lily aphid, *Myzus circumflexus* (Buckt.); and the fox glove aphid, *M. convolvuli* (Kalt.). Aphids were fed for 24 hours or longer on infected Zucchini squash plants and then were transferred in lots of 20 or more to healthy squash test plants for a period of 24 hours. The test plants then were fumigated with nicotine and incubated in the greenhouse for 1 month. From 25 to 85 test plants were used for each aphid species. The virus source plants fed upon by the aphids were demonstrated, by mechanical inoculation, to be good sources of virus. Inoculations to healthy squash usually resulted in 100 per cent infection.

None of the 447 squash plants fed upon by the 9 species of aphids became infected. Earlier tests (7) had resulted in some apparent transmission by means of aphids, but in view of the present results on a large scale, it is considered that the earlier supposed transmission was due to contamination with cucumber mosaic virus.

Watson and Roberts (22) demonstrated that the green peach aphid is much more likely to transmit certain viruses if the aphids are fasted immediately before being fed for a short period on source plants. Since aphids failed to transmit squash mosaic virus after long acquisition feeding periods, additional tests were made in which fasted aphids were used, and the acquisition periods were very short. Green peach aphids were fasted in small glass vials for periods of 1 hour or more and then given feeding periods varying from 15 seconds to 10 minutes on infected squash plants. No transmission was obtained in tests with 150 aphids.

Melon aphids failed to transmit squash mosaic virus when transferred in lots of 25 from infected cucumber plants to healthy cucumber plants in 20 tests. Melon aphids collected on naturally infected squash plants in the field and transferred to healthy squash plants in the greenhouse also failed to transmit the virus. It was concluded from all these tests that aphids are not involved in the field spread of squash mosaic virus.

Five species of leafhoppers and 2 species of plant bugs failed to transmit the virus. These tests included the beet leafhopper, *Circulifer tenellus* (Baker); the mountain leafhopper, *Colladonus montanus* (Van Duzee); the geminate leafhopper, *C. geminatus* (Van Duzee); *Empoasca* sp.; *Aceratagallia* sp.; the squash bug, *Anasa tristis* Deg.; and *Lygus* sp. The insects were fed 1–2 days on infected squash plants and then transferred in lots of 20 to healthy squash plants. A total of 139 squash plants were fed on by these insects, but none of them became infected.

TRANSMISSION OF SQUASH MOSAIC VIRUS BY CUCUMBER BEETLES.—Doolittle's (4) report that cucumber beetles are vectors of cucumber mosaic virus suggested the possibility that cucumber beetles might act as vectors of squash mosaic virus. Tests with the western striped cucumber beetle, *Acalymma trivittata* (Mann.), indicated that it was capable of transmitting the virus. The beetles were fed on infected Zucchini squash plants for 1–14 days, depending on the number of beetles and virus source plants available. The beetles were then given a 1-day feeding period on the test plants.

The results (Table 2) indicate that the western striped cucumber beetle is an efficient vector of the virus. The feeding of single beetles resulted in infections in 36 of 90 plants and that of 2 beetles per test plant in 28 of 32 plants.

The western twelve spotted cucumber beetle, *Diabrotica undecimpunctata* Mann., was a much less efficient vector of the virus than was the striped beetle (Table 2). The results of the tests with both beetles

TABLE 2.—*Transmission of squash mosaic virus from squash to squash by cucumber beetles*

Kind of cucumber beetle	Number of beetles in colony	Number of colonies that caused infections[a]	Number of test plants infected[b]
Western striped	1	5/7	36/90
	2	4/4	28/32
	5	2/4	5/20
	10	2/2	25/27
Twelve spotted	1	1/1	2/25
	10	6/7	22/72

[a] Numerator is number of colonies that caused infections, and denominator is number of colonies tested.

[b] Numerator is number of test plants infected, and denominator is number of test plants used.

indicate either a considerable variation in ability of individual beetles to transmit the virus or in the virus source plants.

ACQUISITION THRESHOLD PERIOD. — Beetles were starved at room temperature for 1, 2, and 3 days and then given short acquisition feeding periods. The results demonstrated that beetles could acquire the virus in a 5-minute feeding period. Seven of 25 beetles that fed 5 minutes on infected squash leaves following 3 days starvation proved to be infective. Virus also was acquired during a 5-hour acquisition feeding and transmitted to healthy squash during the succeeding 5 hours, indicating that the beetles can acquire and transmit the virus in less than 10 hours. Thus any latent period of the virus in the beetle must be less than 10 hours.

RETENTION OF VIRUS BY CUCUMBER BEETLES.—Transmission experiments were conducted to determine 1) how long the cucumber beetles could retain virus when transferred daily to healthy squash plants, and 2) whether the number of beetles used or the length of the acquisition feeding period would influence either virus retention or the number of plants infected.

TABLE 3.—*Retention of squash mosaic virus by individual western striped cucumber beetles transferred daily to healthy squash plants*[a]

	Transmissions[c] by indicated individual insects that had										
	Fed 13 days on source plants								Fed 15 days on source plants		
Transfer[b]	1	2	3	4	5	6	7	8	9	10	11
1	—	+	—	—	—	+	—	+	+	+	—
2	—	—	—	—	—	+	—	+	—	—	+
3	—	+	—	—	—	—	—	—	+	—	—
4	+	+	+	+	—	+	+	+	—	—	—
5	—	+	—	—	—	—	+	—	+	+	—
6	—	—	+	—	—	—	—	—	+	—	—
7	—	+	—	—	+	+	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—	—
9	—	+	—	—	—	+	—	—	—	—	—
10	—	×[d]	+	—	—	—	—	—	—	—	—
11	—		—	—	—	+	—	—	—	—	—
12	—		—	—	—	—	—	—	—	—	—
13	—		—	—	—	+	—	—	—	—	—
14	—		—	—	—	—	×[d]	—	—	—	—
15	—		—	—	—	+	—	—	—	—	+
16	—		—	—	—	—	—	—	—	—	—
17	—		—	—	—	+	—	—	—	—	—
18	—		—	—	—	—	—	—	—	—	—
19	—		—	—	—	—	—	—	—	—	—
20	—[e]		—[e]	—[e]	—[e]	—		—[e]	—	—	—

[a] Twenty insects were used in this test, only the 11 that produced infection are included in table; 8 of 10 at 13 days' feeding and 3 of 10 at 15 days' feeding on virus source plants.

[b] First transfer to healthy squash plants was made immediately following acquisition feeding. Subsequent transfers were at 1-day intervals.

[c] — indicates no infection; + indicates infection.

[d] × indicates transfers discontinued because of loss or death of beetle.

[e] Transfers continued for 28 days with no additional infection.

Twenty western striped cucumber beetles were fed on infected squash plants then transferred daily to healthy squash plants, 1 insect per plant. Eight of the 10 beetles given a 13-day acquisition feeding and 3 of the 10 beetles given a 15-day acquisition feeding acquired and transmitted the virus (Table 3). Individual beetles caused infections in from 1 to 9 squash plants while feeding on a total of 9 to 28 each. There was considerable variation in the number of infections produced by individual beetles. Of the 3 beetles that infected only 1 plant, 2 infected the fourth plant and 1 the seventh plant. The longest period of retention in this experiment was 17 days. Individual beetles infected plants at irregular intervals.

In a second experiment, striped cucumber beetles were given varying acquisition feeding periods of 2, 10, 13, and 15 days to determine whether increased feeding time would increase the number of transmissions or the length of virus retention by the insects. Length of acquisition feeding had no consistent effect on numbers of transmissions (Table 4). Those insects fed on source plants for 13 days caused an unusually high number of infections, otherwise all groups caused approximately the same number of infections. There was, however, increased retention with increased length of acquisition feeding. A 2-day acquisition feeding resulted in a 2-day retention period, the 10-day feeding in an 8-day retention, the 13-day feeding in a 17-day retention, and a 15-day feeding in a 15-day retention. More plants became infected during the

TABLE 4.—*Transmissions of squash mosaic virus by single western striped cucumber beetles given acquisition feeding periods of 2, 10, 13 or 15 days and then transferred daily to healthy squash plants*

	Transmissions[a] by insects given acquisition feeding periods of			
Transfer[b]	2 Days	10 Days	13 Days	15 Days
1	8/20	2/10	3/10	2/10
2	1/17	2/10	2/10	1/10
3	0/15	1/10	1/10	1/10
4	0/13	1/10	7/10	0/10
5	0/12	0/9	2/10	2/10
6	0/10	0/9	1/10	1/10
7	0/8	1/9	3/10	0/10
8	0/8	1/8	0/10	0/10
9	0/7	0/8	2/10	0/10
10	0/6	0/8	1/9	0/10
11	0/6	0/8	1/9	0/10
12	0/6	0/8	0/9	0/10
13	0/6	0/8	1/9	0/10
14	0/5	0/8	0/8	0/10
15	0/5	0/8	1/8	1/10
16	0/5	0/8	0/8	0/10
17	0/4	0/8	1/8	0/10
18	0/4	0/8	0/8	0/10
19	0/4	0/8	0/8	0/10
20	0/4	0/8	0/8	0/10

[a] Numerator indicates number infected and denominator number of plants the beetles fed upon.

[b] First transfer to healthy squash plants was made immediately following acquisition feeding. Subsequent transfers were at 1-day intervals.

first few days than during subsequent feedings.

A comparison was made of the retention of squash mosaic virus by the western striped and the western spotted cucumber beetle. Groups of 5 beetles of each species were given varying acquisition feeding periods followed by daily serial transfer to healthy squash plants (Table 5). These results are similar to those previously shown (Tables 3, 4) in that they fail to indicate any correlation between length of acquisition feeding period and number of transmissions but do show a rapid exhaustion of virus charge. There appears to be no apparent difference in the retention pattern of the virus by these 2 beetles. The spotted cucumber beetle retained the virus for 19 days following a 16-day acquisition feeding and for 20 days after a 13-day acquisition feeding. The transmission pattern was rather unusual in the latter case in that the feeding of the group of 5 insects resulted in no infections in the 100 plants fed upon during the first 10 days but infections did occur in plants fed upon during days 11, 12, 13, 14, 15, and 20. The western striped cucumber beetle retained the virus for a period of 18 days following a 6-day acquisition feeding. Several groups of 5 beetles transmitted the virus during the first 3 days, then failed to do so for 13 days, but did so again on days 17 and 18. The irregular transmission of the virus and the long periods between infections are difficult to interpret. These are also known to occur in the transmission of the curly top virus by the beet leafhopper (5). The western spotted cucumber beetle appears to be slightly less efficient than the western striped cucumber beetles.

TABLE 5.—*Retention of squash mosaic virus by groups of 5 western striped cucumber beetles and of the western twelve spotted cucumber beetles transferred daily to healthy squash plants*[a]

	Striped cucumber beetles fed on source plants for				Spotted cucumber beetles fed on source plants for		
Transfer[b]	1 Day	6 Days	10 Days	13 Days	3 Days	13 Days	16 Days
1	2/10	5/10	7/10	4/10	2/10	0/10	4/10
2	6/10	4/10	1/10	4/10	0/10	0/10	0/10
3	5/10	3/10	2/10	2/10	0/10	0/10	2/10
4	0/10	0/10	2/10	0/10	0/10	0/10	0/10
5	0/10	0/10	0/10	0/10	0/10	0/10	0/10
6	0/10	0/10	2/10	0/10	0/10	0/10	2/10
7	0/10	0/10	0/10	0/10	0/10	0/10	0/10
8	0/10	0/10	0/10	0/10		0/10	0/10
9	0/10	0/10	0/10	0/10		0/10	0/10
10	0/10	0/10	0/10	0/10		0/10	0/10
11	0/10	0/10	0/10	0/10		1/10	0/10
12	0/10	0/10	0/10	0/10		2/10	0/10
13	0/10	0/10	0/10	1/10		3/10	0/10
14	0/10	0/10	0/10	0/10		1/10	0/10
15	0/10	0/10	0/10	0/10		2/10	0/10
16	0/10	0/10	0/10	0/10		0/10	1/10
17	0/10	1/10	0/10	0/10		0/10	0/10
18	0/10	1/10	0/10	0/10		0/10	0/10
19	0/10	0/10	0/10	0/10		0/10	1/10
20	0/10[c]	0/10[c]	0/10[c]	0/10[c]		1/10[c]	0/10[c]

[a] Numerator indicates number plants infected and denominator number of plants the beetles fed upon.

[b] First transfer to healthy squash plants was made immediately following acquisition feeding. Subsequent transfers made at 1-day intervals.

[c] Transfers continued for 24 days with no further infection.

Long retention of the squash mosaic virus under experimental conditions suggested the possibility that the virus might overwinter in the bodies of the beetles under natural conditions. In order to obtain evidence on this question, adults of the western striped cucumber beetles were collected in February on weeds not known to be susceptible to the virus. The beetles were collected in an area where no squash plants were observed at the time. The overwintering beetles were transferred to healthy squash seedlings in the greenhouse. Several transmissions resulted, demonstrating that the adult beetles were carrying the virus and that the virus may have overwintered within the bodies of the insects.

The fact that the beetles retained the ability to transmit virus for a period of 20 days indicates other than mere mechanical transmission of the virus by externally contaminated mouthpart surfaces as has been assumed in the transmission of other viruses by mandibulate insects.

REGURGITATION.—Markham and Smith (14) have indicated that mandibulate insects probably transmit yellow turnip mosaic virus through the process of regurgitation. Western striped cucumber beetles feeding on squash plants infected with squash mosaic virus were removed and confined singly in small gelatine capsules. They were then anesthetized with ether. A few moments after exposure to the ether, the majority of beetles regurgitated a clear liquid of a greenish amber color. This liquid was absorbed on a cotton swab on the end of a pin. The swab was then immersed briefly in a drop of distilled water, touched to Carborundum powder and rubbed on test plants. Of 10 squash plants thus inoculated, 8 became infected. The results suggest that regurgitated fluid is a probable source of the squash mosaic virus in the transmission by the cucumber beetles.

RECOVERY OF VIRUS FROM THE BODIES OF CRUSHED BEETLES.—In general, inoculation of plants with juice of crushed infective insects have failed to result in infections. Hamilton (10) showed that the addition of juice from crushed green peach aphids to inoculum containing henbane mosaic virus rendered the mixture noninfective. Black (1) concluded that the apparent general inhibiting action of insect juices upon the infectivity of plant viruses is one reason for general failure to successfully inoculate plants with the juice of viruliferous insects.

Squash mosaic virus was readily recovered from the crushed bodies of the western striped cucumber beetle taken from diseased plants. Fifty beetles were ground in a mortar with a pestle, and 2 ml of distilled water was added. Ten healthy squash plants were inoculated with this extract, and all developed typical symptoms of squash mosaic. Six of 10 squash inoculated with the crushed bodies of infective western twelve spotted cucumber beetles became infected.

The virus was recovered also from the crushed bodies of infective western striped cucumber beetles following a 5-day feeding period on healthy squash plants. Three of 10 plants inoculated with the crushed bodies of these insects became diseased, but juice from beetles crushed 12 days after transfer from infected plants was not infectious.

Attempts to recover squash mosaic virus from the crushed bodies of the potato aphid, *Macrosiphum solanifolii* (Ashm.), failed.

Virus was recovered also from naturally infective beetles. Both striped and spotted cucumber beetles were collected in melon fields. They were first fed on healthy squash seedling for 1 day, and a high percentage of test plants became diseased. The insects were then crushed, and the juice used to inoculate healthy squash plants. All of these became diseased.

Recovery of virus from the blood of beetles.—Recovery of squash mosaic virus from the crushed bodies of beetles raised the question of the location of the virus within the body of the insect. Attempts therefore were made to recover squash mosaic virus from the blood of cucumber beetles. Striped and spotted cucumber beetles that had fed on infected squash plants were etherized. In the first experiment, the 2 hind legs of each beetle were pulled off and light pressure applied to cause a drop of blood to ooze out through the wound. Blood was picked up on a dissecting scalpel and rubbed on a cotyledon of a squash plant. Two insects were used for each plant; 1 insect for each cotyledon. Twenty-five plants were inoculated in the manner described, and 3 of these developed typical symptoms of squash mosaic.

In a second experiment, a shallow incision was made on the abdomen with a dissecting scalpel. Light pressure was applied, causing a drop of blood to ooze out. This blood was rubbed on a cotyledon of a squash plant. Two of 25 plants inoculated developed symptoms. These experiments demonstrated the presence of active virus in the blood of infective beetles.

Recovery of virus from the feces of beetles.—Walters (21) demonstrated that tobacco mosaic virus, potato ringspot virus, and tobacco ringspot virus, after passage through the alimentary canal of grasshoppers, was present in an active state in naturally voided feces. In the present work, squash mosaic virus was found in the feces of cucumber beetles.

Twenty-five infective western striped cucumber beetles were transferred from infected squash plants to sterilized test tubes and kept there for 1 hour. The test tubes were then washed with 1 ml of sterilized distilled water by shaking thoroughly for several minutes. The water containing suspended feces was inoculated into 5 healthy squash plants. The beetles that had been removed and transferred to healthy squash plants were returned to sterilized test tubes for fecal deposition at the end of 2, 5, 7, and 10 days.

The results of 3 experiments with the western striped cucumber beetle showed that 15 of 15 squash plants inoculated with feces from beetles taken directly from infected plants developed symptoms of squash mosaic. Eleven of 15 squash inoculated with feces deposited by beetles that had been confined for 2 days on healthy plants became diseased, 1 of 15 at 5 days, and 2 of 15 at 7 days. None of the plants inoculated with the feces of beetles kept 15 days on healthy plants developed symptoms of the disease.

Inoculations with feces collected from infective western twelve spotted cucumber beetles kept for various periods of time in healthy plants resulted in infections as follows: immediately following infective feeding, 10 plants infected of 10 plants inoculated; 2 days, 3 of 10 plants; 5 days, 1 of 10 plants; 7 days, 1 of 10 plants; and 10 days, 2 of 10 plants.

The results of these experiments indicate that the feces of the cucumber beetles are an excellent source of virus and that active virus is retained in the alimentary tracts for 7–10 days. The beetles were not observed to regurgitate during these experiments, but it is possible that the results may have been influenced by some regurgitation.

Transmission of cucumber mosaic virus by cucumber beetles.—Doolittle (4) reported cucumber mosaic virus transmission to cucumber by both aphids and cucumber beetles, but apparently no confirmation of his results with cucumber beetles has been published. Therefore, both the western striped and western spotted cucumber beetles were tested as possible vectors of cucumber mosaic virus. Several strains of western cucumber mosaic virus were used in these tests. The beetles were fed from 1 to 7 days on infected Zucchini squash plants and then transferred in groups of 2, 5, and 10 to healthy squash plants. The western striped cucumber beetle failed to transmit the virus to any of the 307 healthy plants on which they were fed. Included in these tests was 1 trial with common cucumber mosaic, in which beetles were fed 1 day on infected squash plants and then transferred in groups of 5 to 23 healthy squash plants. None of these test plants developed symptoms of common cucumber mosaic. Lots of 5 and 10 western spotted cucumber beetles failed to transmit cucumber mosaic virus to any of the 80 squash plants on which they fed.

Failure of the 2 species of western cucumber beetles to transmit cucumber mosaic virus to squash indicates the need for verification of the earlier report by Doolittle (4) that the 2 closely related eastern species of beetles are vectors of common cucumber mosaic virus.

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THE VALIDITY OF BINOMIALS IN THE LEAF RUST FUNGUS COMPLEX OF CEREALS AND GRASSES[1]

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SUMMARY

It is recommended that *Puccinia elymi* West. be considered as a distinct species because of its multiseptate teliospores. The oldest valid name applied to forms with 2-celled teliospores of the leaf rust fungus complex of grasses and small grains is *Puccinia recondita* Rob. ex Desm. described on rye in 1857, thus antedating *Puccinia rubigo-vera* Wint. of 1882.

The fungus that causes leaf rust or brown rust of grasses, including the small grains, is a variable and plurivorous species to which many names have been applied. Mains (7) in 1933 lists 32 binomials in *Puccinia* as synonyms of what he treats as *Puccinia rubigo-vera* (DC.) Wint., and several species have since been published that doubtless should be referred to the leaf rust fungus complex. Since the International Code of Botanical Nomenclature (6) now specifically excludes names based on the uredial stage, *Uredo rubigo-vera* DC. (1815) can no longer serve as the basonym. *Puccinia rubigo-vera*, if used at all, must date from Winter's (10) description (1882) of the telial stage, and there are intervening names that prevent such usage.

On nomenclatural grounds there are 2 validly published names, both with types extant and bearing telia, that must be considered: 1) *Puccinia elymi* Westendorp (1852) and 2) *Puccinia recondita* Roberge ex Desmazieres (1857).

P. elymi, collected by M. Louis Landzweert "dans les dunes d'Ostende" on *Elymus arenarius* L. and issued as No. 291 in Westendorp and Wallays Pl. Crypt. Belge, has teliospores that are predominantly 3- or 4-celled (Fig. 1, A) and only rarely 2-celled. This rust fungus subsequently (1889) was used by Lagerheim (5) as the basis of the genus *Rostrupia*. Westendorp's (8) description reads "Groupes brunâtres,

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FIG. 1. A) The multiseptate teliospores characteristic of *Puccinia elymi* West. described on *Elymus arenarius* (from Pl. Crypt. Belge No. 291). B) Two-celled teliospores of *Puccinia recondita* Roberge ex Desm. described on rye (from Pl. Crypt. France ser. 2, No. 252). ×800.

devenant presque noirs, allongés, aplatis tachant l'épiderme en noir grisâtre et restant presque toujours recouvertes par celui-ci, quoiqu'il se déchire au centre des pustules et longitudinalement. Pédicelle court, gross et transparent, supportant des sporidies fusiforms, biloculares." Although Westendorp described the teliospores as "biloculares," Lagerheim (5) has suggested that Westendorp mistook the lower cell for the pedicel. In any case, in accordance with Article 13 of the International Code (6), epithets are based on type specimens, and since the original collection is rostrupioid, the name (*P. elymi*) can apply only to a rust fungus having such spores. Since this morphological feature is distinctive, we propose, as have also Mains (7), Wilson and Bisby (as *Rostrupia elymi*) (9), and Hylander, Jørstad, and Nannfeldt (4), among others, that *P. elymi* be considered as a species related to but distinct from the leaf rust organism which has 2-celled teliospores.

P. recondita Rob. ex Desm., the next name as regards priority, was collected by Roberge in France on "foliis languescentibus Secalis" and was issued as No. 252 in Desmazieres Pl. Crypt. France ser. 2. This specimen has 2-celled teliospores (Fig. 1,B) and corresponds to the usual concept of the leaf rust fungus. The description, published by Desmazieres (3), reads: "P. maculis luteolis. Acervis amphigenis, ovalibus, oblongis, dein linearibus, saepe confluentibus epidermide sero rumpenti fectis. Sporidiis elongato-clavatis, medio vix constrictis, loculo superiore fusco, obtuso, subrotundato seu ovali; inferiore subpellucido, elongato, sensim tenuato; stipite albo, brevissimo." The label of No. 252 as to host reads "sur les feuilles languisantes du Seigle." Interestingly enough, this name, so obviously applying to the fungus causing leaf rust of rye, seems never to have been given consideration although cited in synonymy by Arthur (1) and Cunningham (2). The acceptance of *P. recondita* Rob. ex Desm. as the name at least for leaf rust fungus of rye, is obligatory.

Thus, *P. recondita* becomes the oldest valid binomial applicable to leaf rust fungi of the "*rubigo-vera* complex" if *P. elymi* is treated as a distinct species as recommended above. Other names, such as *P. rubigo-vera* Wint. (1882), *P. perplexans* Plowr. (1885), *P. persistens* Plowr. (1889), *P. dispersa* Eriks. (1894), and *P. triticina* Eriks. (1899) are later synonyms.

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PHYSIOLOGICAL ASPECTS OF VERTICILLIUM WILT[1]

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SUMMARY

In tomato plants infected with *Verticillium albo-atrum* Reinke & Berth., transpiration was inhibited preceding and accompanying symptom expression. Vascular dysfunction in the form of browning and vessel blockage was evident, leading to the conclusion that wilt was the result of interference with water conduction. Culture fluids of Verticillium contained a heat-labile vascular-browning factor. Enzyme assays of the culture fluids showed the presence of a polygalacturonase and very little pectin methyl esterase. Verticillium differed from the previously studied Fusarium in that the latter produced pectin methyl esterase and depolymerase.

Verticillium albo-atrum Reinke & Berth. invades the roots and affects the vascular system of plants in much the same way as do the vascular Fusaria. There are, however, certain distinct differences, both in host range and in responses of the diseases to environmental conditions. Verticillium symptoms tend to increase with increases in mineral nutrient concentrations (19), whereas symptoms of Fusarium wilt decrease (18). High light intensity is said to favor Verticillium wilt (14), whereas plants grown under low light conditions are more susceptible to Fusarium wilt than are plants grown under high light conditions (7). *V. albo-atrum* has a wide host range, whereas the vascular Fusaria are host-specific. For these and other reasons it became of interest to examine the physiological mechanisms involved in Verticillium wilt in comparison with those in Fusarium wilt.

The cause of wilting in Verticillium-infected plants has received much study, but it is still poorly understood. An early theory held that mycelial growth plugged the vessels (2). Other workers, notably Czarnecki (3), maintained that wilt resulted from mechanical obstruction of vessels by gums and tyloses. The systemic toxin theory of wilting has been supported by Dowson (5) and many others. Especially notable in the early work is that of Bewley (1), who considered the possibility of extracellular enzymes as a factor in the disease syndrome. Although he demonstrated the presence of several hydrolytic enzymes in culture fluids, he was not able to find pectic enzymes. Dufrenoy's nitrite theory (6) has been reexamined recently by Green (9), who found no supporting evidence. The present work also is concerned largely with the explanation of wilting in diseased plants.

MATERIALS AND METHODS.—The tomato variety Bonny Best was used in all experiments. Plants were grown in the greenhouse in sand watered with a mineral nutrient solution. When cuttings were required, they were handled as they were in earlier work with Fusarium wilt (15). Six- to 8-in. cuttings were taken from 5- to 7-week-old plants carefully selected for uniformity. When diseased material was required, plants were removed from the sand, inoculated by dipping the roots in a spore-mycelium suspension, and reset in sand or soil at 20°C.

Two isolates of *V. albo-atrum* were used. The isolate used for inoculating tomato plants and for enzyme assays was obtained from Walter F. Virgin, California Packing Corporation, San Francisco, California, and is herein designated as T-16. The other isolate, used in certain laboratory experiments, originally came from diseased chrysanthemum and was obtained from C. Olsen of Yoder Brothers, Inc., Barberton, Ohio. Both isolates produced microsclerotia on culture media. Cultures for inoculum were grown in Czapek's solution on a continuous shaker at 25°C. After 10 days' growth, the cultures were filtered, the filtrate discarded, and the fungus growth was homogenized in water with a Waring Blendor.

The effect of infection on transpiration of growing plants was determined by the technique developed for Fusarium wilt (15). Inoculated and control plants 3.5 in. high were set in vermiculite in waxed paper boxes and sealed (Fig. 1). Care was taken to have exactly the same moisture content in each box. Water loss was determined twice daily by measuring the amount of water, added through a small glass funnel, required to bring the boxes back to the original weight. At the beginning of the experiment, plants were carefully selected for uniformity of size and appearance. Leaf areas of all experimental plants were determined at the end of the experiment by tracing leaf outlines on paper and measuring these with a planimeter. Water loss per unit area was determined.

The effect of infection on xylem conductivity was studied by use of a 1 per cent aqueous solution of eosin. Conduction of the solution by petioles was determined on plants *in situ* by cutting off the end of the petiole and placing in the dye the cut end of the section attached to the stem. Conduction in main stems

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FIG. 1. Plant in sealed paper box for determination of water loss by weighing.

was studied by allowing transpirational pull to take up dye solution through the basal ends of cuttings. In other cases, rubber tubing was fixed to the tops of stem sections, and dyes were drawn through by vacuum. Dye distribution in all cases was checked by microscopic examination of free-hand sections of the stems or petioles.

Culture fluids, used for treating cuttings and for enzyme assays, were obtained from bran cultures. The fungus was grown on sterilized moist bran (25 g of wheat bran plus 25 ml water) at 25°C for 5–7 days, unless otherwise stated. After the desired growth had occurred, 200 ml of sterile water was added, the preparation was agitated gently, allowed to stand overnight, then filtered through paper and centrifuged at approximately 4000 rpm for 60 minutes to remove mycelial fragments and spores.

Methods used to assay extracellular enzymes in culture fluids were the same as those used by Gothoskar et al (8). Pectin methyl esterase (PME) activity was assayed by alkali titration to determine the mg of methoxyl groups hydrolyzed from pectin solutions. Polygalacturonase (PG) activity was assayed by a modification of the method of Jansen and MacDonnell (11) based on the increase in reducing groups resulting from the breakdown of the polypectate molecule. This method is most accurate in the later stages of hydrolysis. In the earlier stages, reduction in viscosity is more sensitive. Viscosity determinations were conducted at approximately 30°C with conventional Ostwald viscosimeters. Methoxyl determination of pectin was made according to the procedure of Hinton (10). β-glycosidase activity was assayed by thiosulfate-iodine titration of reducing groups liberated from salicin.

EXPERIMENTAL RESULTS.—*Effect of infection on transpiration.*—Water loss by inoculated plants was compared with loss in healthy plants under greenhouse conditions at 20°C (± 1.5°) in March. Twenty-five small tomato plants were carefully selected for uniformity, and 5 were used for leaf-area measurements at the beginning of the experiment. Ten inoculated and 10 noninoculated plants were set in vermiculite, sealed in waxed boxes (Fig. 1), and randomized on a table. The experiment was continued for 18 days, after which leaf areas were taken.

First symptoms were seen on the eighth day, when 3 plants each had 1 wilted leaf. After 13 days, 8 plants showed wilt, and 9 had yellowing of lower leaves. The affected leaves flagged during the day but recovered at night until the fifteenth day, when several failed to recover. At no time during the experiment were more than 30 per cent of the leaves wilted, but near the end of the experiment all inoculated plants except one had yellowed lower leaves and were stunted.

Water loss by inoculated plants is expressed as the ratio of water loss of inoculated plants to that of the controls (Fig. 2) because of the large day-to-day variations resulting from differences in humidity, light, and other conditions that were not under control in the greenhouse. This variation in water loss ranged from 42 to 130 ml per 1000 sq. cm. of leaf area per day. Water losses recorded on the third through the sixth

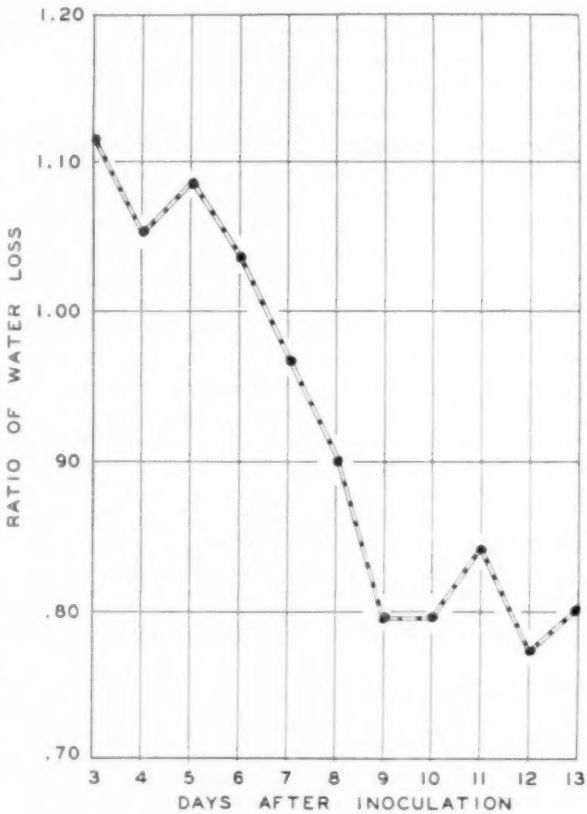

FIG. 2. Water loss in Verticillium-infected plants expressed as a ratio of water loss in infected plants to that in healthy plants under the same conditions.

day were greater for the inoculated plants than for the controls, but the differences were not statistically significant at the 5 per cent level. Just prior to and during symptom development, the infected plants transpired at a significantly lower rate than did the controls. The average transpiration values may not give a true picture because of differences in rate of disease development, hence each plant was considered separately and its water loss compared with the average of control plants. Without exception, there was a decrease in water loss before and during wilting.

These data indicate that wilting results from decreases in water supply to leaves, which could be caused by vascular dysfunction. They do not support the view that wilt is the result of toxic action that causes increased water loss in leaves. Another point of interest is that interference with water loss by Verticillium in this case was much less drastic than that caused by Fusarium (15).

In diseased plants that were allowed to take up the dye through petioles *in situ*, there was slight to no conduction of dye through the petioles of wilted leaves. In healthy plants, the dye was carried well down the main stem within 30 minutes. In diseased cuttings that were allowed to take up dye by transpirational pull, no more than 2 of the 6 main bundles of the stem were completely blocked, but some vessels of all the bundles failed to carry the dye. Here again all of the petiole vessels were blocked in leaves with severe wilt, but only part of them were blocked in leaves with mild wilt. Essentially the same results were obtained when dyes were forced through stem sections. It was apparent that *V. albo-atrum* caused some vascular dysfunction, but the vessel blocking was not nearly so striking as that caused by Fusarium (15).

Effects of culture filtrates on cuttings.—Tomato cuttings were treated with culture fluids of isolate T-16 to determine whether or not *V. albo-atrum* produces a heat-labile vascular browning and wilting factor similar to that found in Fusarium filtrates (8, 15). Portions of the culture fluid, prepared as described previously, were heated for 10 minutes in a water bath at 100°C. The heated and unheated solutions were diluted with water, and cuttings were allowed to take up the diluted solutions for 2 days. These were then placed in water until they were rated on the fourth day for vascular browning and for wilt on the 0 to 4 scale used in previous work (8). Vascular browning developed in cuttings placed in unheated solutions, even when the original solution was diluted 1:16 with water but did not develop in those placed in heated solutions (Table 1). The finding that the factor is heat-labile suggests that it may be similar to that produced by Fusarium (8). The fact that heating did not destroy the ability of culture solutions to cause wilt in cuttings (Table 1) may not be significant because many materials of low toxicity are expected to be present in high concentration in such fluids. Many of these, such as polysaccharides, are heat-stable and will cause a wilt that probably cannot be associated with disease development (15).

TABLE 1.—*Wilt and vascular browning of tomato cuttings exposed to heated and unheated culture fluids*[a]

Dilution and treatment	Culture fluid taken up (ml)	Wilt index[b]	Vascular browning index[c]
Unheated 1:4	4.5	2.3	2.0
Unheated 1:8	9.0	2.0	1.3
Unheated 1:16	14.0	1.5	1.0
Heated[d] 1:2		2.0	Trace
Heated[d] 1:4		2.5	Trace
Heated[d] 1:8		2.0	0
Heated[d] 1:16		1.0	0
Unheated 1:2		3.0	3.0
Unheated 1:4		3.0	1.5
Unheated 1:8		2.5	1.0
Unheated 1:16		2.0	1.0
H_2O Check	30	0	0

[a] All values are the averages for 4 cuttings each.
[b] 0 = No wilt, 4 = death of cutting.
[c] 0 = No browning, 4 = vascular browning to the end of the stem.
[d] Heated at 100°C for 10 minutes.

No spores could be detected in the centrifuged fluid by microscopic examination, but the possibility remained that a few contaminating spores might be present and might account in part for the symptoms observed in cuttings after 4 days. As a check on this possibility, spores were obtained from cultures and were added in several low concentrations to water and to the centrifuged culture fluids at several dilutions. In no case did the addition of spores to the fluids give increases in vascular browning. It was concluded that if contaminating spores were present, they were not an important factor.

Pectic enzyme activity of the culture filtrate.—The thermolabile nature of the vascular browning factor suggested that enzymes similar to those found for Fusarium might be involved. Culture fluids were therefore assayed for pectic enzyme activity. PME activity was determined with 2 strains of the fungus, using cultures from 5 to 21 days old and incubation temperatures for growth from 16° to 24°C. Several media were used, including moist bran, bran plus dextrose and pectin, and synthetic media containing pectin and dextrose. In all cases, PME activity either was not detectable or was very low. Nevertheless, when bran culture filtrates were added to pectin or sodium polypectate solutions, viscosities of both solutions were reduced very rapidly (Fig. 3). The rate of reduction in viscosity with sodium polypectate was 3–4 times as fast as that with pectin, suggesting a more rapid breakdown of the sodium polypectate than of pectin. This was confirmed using the reductometric method of assay wherein the liberation of reducing groups was taken as a measure of enzyme activity (Fig. 4).

As the culture filtrates possessed very little PME activity but still hydrolyzed pectin, it was considered possible that another enzyme, a pectin depolymerase as described by Wood (20) and Seegmiller and Jansen (16), was involved. The pectin used in the ex-

periment, however, had a methoxyl content of 8.75 per cent and was consequently only about 50 per cent esterified. Therefore, the breakdown of pectin could be the result of action by this enzyme on the unesterified portion, which resembles polypectate. To check this possibility, a highly esterified pectin with a methoxyl content of 14.8 per cent was prepared by the method of Morell et al (12, 13). When this pectin was used as substrate, a very slight reduction in viscosity was observed on incubation with the culture filtrate (Fig. 3). When this pectin was deesterified with a purified PME from tobacco to a methoxyl content of 7.2 per cent and then incubated with Verticillium culture solution, an appreciable reduction in viscosity was observed (Fig. 3). From these results it is apparent that the substrate of this Verticillium enzyme is polypectate and not pectin.

To determine the extent of hydrolysis of sodium polypectate by the culture filtrate, suitable aliquots of the reaction mixture were removed at intervals of 0, 1, 2, 3, and 7 days and examined for the presence of monogalacturonic acid by means of paper chromatography (8). The presence of monogalacturonic acid could be readily demonstrated at the end of 24 hours and at all later intervals. In addition to that characteristic of monogalacturonic acid, 2 spots having lower R_f values than monogalacturonic acid also appeared in all chromatograms of samples removed at the end of 7 days. Apparently, intermediate oligogalacturonides accumulate in addition to the end product, monogalacturonic acid.

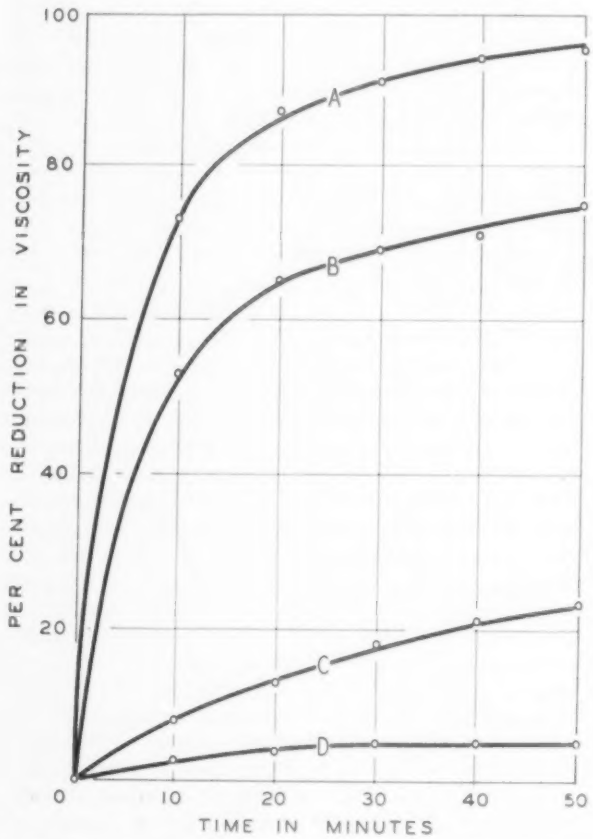

FIG. 3. Effect of the degree of esterification of pectin on polygalacturonase activity of Verticillium culture filtrates. A) Sodium polypectate with 0.27 per cent methoxyl content. B) Pectin with 8.75 per cent methoxyl content. C) Esterified pectin deesterified to a methoxyl content of 7.26 per cent. D) Esterified pectin with a methoxyl content of 14.8 per cent.

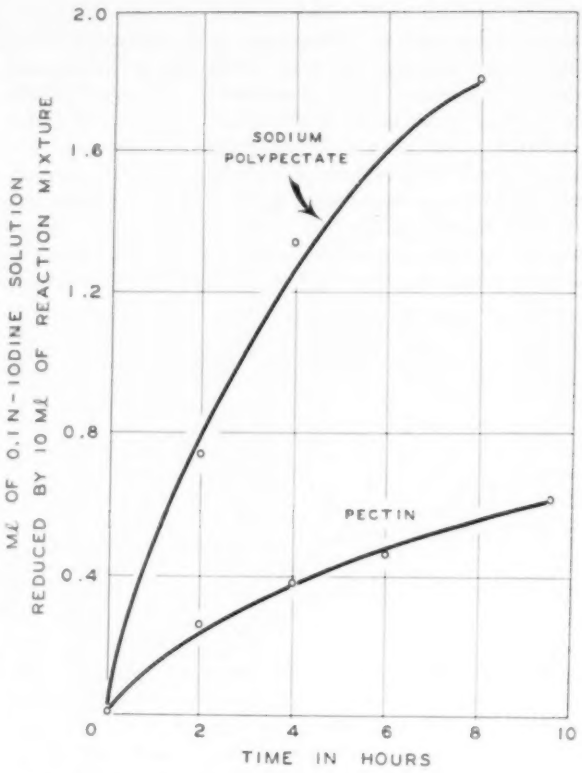

FIG. 4. Rate of liberation of reducing groups from pectin and sodium polypectate solutions by Verticillium culture filtrates. The reaction mixture contained 75 ml of 1 per cent substrate solution and 25 ml of culture filtrate. Ten-ml aliquots were removed at time intervals indicated, and their iodine reducing values were determined. Increase in reducing value over samples removed at zero time was plotted against time.

The action of β-glycosidase has been suggested as a possible source of phenols that appear to have a role in the disease syndrome of Fusarium wilt (4). Accordingly, bran culture fluids of Verticillium were assayed for this enzyme. A definite but low activity for β-glycosidase was found. The role of this enzyme in the disease syndrome is still undetermined.

DISCUSSION.—Tomato plants infected with *V. albo-atrum* often wilt partially during the day and recover at night when transpiration decreases. This alone is circumstantial evidence that wilt results from some interference with water supply. Lower water loss from infected plants than from healthy plants is further circumstantial evidence.

A reduced water supply to leaves could result from

vascular dysfunction or from some interference with water intake by roots. Since only 1 or 2 leaves may show wilt, and the whole plant seldom shows general flagging, local vascular dysfunction seems to be the more likely explanation. Experiments with dye conduction in the xylem support this hypothesis. In the plants examined, however, complete xylem blocking did not occur in more than ⅓ of the large bundles of the stem. This degree of vessel blocking probably would not cause wilt, considering Dowson's (5) experiments in which he notched stems in an unsuccessful attempt to reproduce unilateral wilting. Vascular blocks in the main stem, then, may be much less important than blocks that occur in petioles. As was found in Fusarium wilt (15), vessels in petioles of wilting leaves were blocked in all cases. Also, unilateral wilting of leaflets on 1 leaf can be explained by the vascular dysfunction hypothesis, but is difficult to understand on the basis of systemic toxins. It should be emphasized, however, that vessel blocking is a satisfactory explanation only for wilt; other aspects of the disease syndrome may be caused by other factors that are not considered in this work.

Data on water relations suggest that *V. albo-atrum* may affect tomato plants by a mechanism similar to that of Fusarium (15). Both fungi cause decreased transpiration, but under the conditions used Verticillium appeared to be less effective in reducing water loss. Correlated with this, vascular dysfunction was found to occur in both cases, but again Fusarium had a more decisive effect. Finally, under conditions of these experiments, vascular browning and especially wilting were more severe in Fusarium-infected plants than was the case with Verticillium.

Such considerations led to an examination of Verticillium for hydrolytic enzymes similar to those produced by Fusarium, which are thought to be important in disease development. A heat-labile vascular browning factor was found in culture solutions of both; however, assays for pectic enzymes showed 1 striking difference between the 2 fungi. Fusarium produced in culture much pectin methyl esterase and pectin depolymerase (8), whereas Verticillium produced polygalacturonase but very little pectin methyl esterase. The significance of the difference is unknown, but it is striking enough to warrant further study. Although these experiments do not conclusively prove that the pectic enzymes are involved in the expression of disease symptoms in Verticillium wilt, they provide good supporting evidence that such enzymes are involved. The presence of pectic enzymes has been shown by Waggoner and Dimond (17) in plants with Fusarium wilt.

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EFFECT OF SOME SOIL FACTORS ON EFFICIENCY OF FUNGICIDES IN CONTROLLING RHIZOCTONIA SOLANI [1]

M. Rushdi and W. F. Jeffers [2]

SUMMARY

Tests were made of 7 soil factors on 4 fungicides: Puratized Agricultural Spray, Vancide 51, Dithane D-10, and Acti-dione. Response of these fungicides was measured as percentage of inhibition of growth of *Rhizoctonia solani* in soil.

Soil texture had a significant effect on activity of fungicides in the soil as shown by decreased growth as the amount of sand in the soil was increased. Activity of Dithane D-10, Puratized Agricultural Spray, and Vancide 51 increased as acidity of the soil was reduced whereas the opposite was true for Acti-dione. The type and amount of organic matter also affected activity of fungicides. A carbohydrate (starch) caused a reduction, whereas a protein (casein) showed no effect. One per cent clover hay or wheat straw in soil reduced activity of Dithane D-10 and Puratized Agricultural Spray but not that of Vancide 51 or Acti-dione. A 5 per cent level of clover hay reduced fungicidal activity of all the compounds except Vancide 51.

Fungicidal activity was not changed by any of 3 soil moisture levels. Likewise, an increase in temperature from 21° to 30° C had no effect although it favored linear growth of *R. solani* in soil free of fungicides.

These results indicate that some soil factors controlling fungicidal activity are soil texture, pH, and organic matter content. It appears that these factors may be involved in regulation of adsorptive power of the soil. The degree of adsorption by different soils probably is the most important factor affecting activity of soil fungicides.

Despite the common application of fungicides to soil for control of soil-borne pathogens, there appears to be relatively little literature dealing directly with the effect of soil factors on activity of such materials. One of the earliest reports relating to this subject was that of Jones (12), who found that soil moisture and temperature markedly affected seed-treatment tests with organic mercury fungicides. The effect was primarily on seed germination, however, rather than on the chemicals. In other seed treatment tests, Forsberg et al (5) noted much variation in fungicidal efficiency which they attributed to soil conditions. Another indirect effect of soil factors was reported by Huber and Baur (11), who found that rate of decomposition of calcium cyanamide governed duration of its toxicity to apothecia of *Sclerotinia fructicola*. Rate of decomposition in soil was likewise reported (3) as being of much importance in governing effectiveness of organic mercury compounds. There was indirect evidence that soil adsorption is of importance in determining fungicidal activity of these compounds.

Adsorptive power of clay and organic matter has been shown by Gottlieb and other workers (6, 7, 14, 16) to be of significance in regulating activity of antibiotics in soil. In other work with antibiotics, Gregory et al (8) reported that activity was not greatly affected by soil type or reaction. Several reports (1, 2, 9, 10, 13) have described some effect of soil factors on fungicidal activity, but it is obvious that there is need for much work on this important problem.

MATERIALS AND METHODS.—Use of Christiana soil[3] made it possible to vary soil texture by adding washed white sand. In all tests, the soil was dried at room temperature for 5 days, ground with a meat grinder, and then passed through a 50-mesh screen. In texture tests, sand amounts were varied, and in moisture tests, amounts of water were varied. In all other tests, 60 per cent by weight of sand was added to the soil, then sufficient water or fungicide solution was added to bring the soil moisture level to 15 per cent.

A modification of a technic described by Norton (15) was used to measure linear growth of fungi in soil. A section of glass tubing 18 cm long with an inner diameter of 1.3 cm was marked at 4.5 cm from 1 end and again at the center. A cotton plug was inserted until even with the mark near the end. Half of a 15-g sample of soil was poured on top of the cotton plug. This filled the tubing to the center mark. The other end of the tubing was then plugged with cotton. The remainder of the soil was kept in a cotton-plugged test tube. Both tubes were autoclaved for ½ hour at 15 lb. pressure. After 48 hours, a 7-mm agar disc from a 48-hour-old Petri plate culture of *Rhizoctonia solani* Kühn was placed on top of the soil in the glass tubing. The soil in the test tube was then poured on top of the inoculum.

When finally prepared, the tubes were kept for 4 days at room temperature (about 25°C) in a metal box containing wet paper towels to reduce water loss. Under these conditions, mycelium grew through the soil as well as between the soil and the tubing wall.

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[2] This publication is based on a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, University of Maryland.

[3] An analysis of Christiana soil, after decomposition of organic matter, was 74.8 per cent clay, 18.7 per cent silt, and 6.5 per cent sand. The pH of this soil was 4.6.

A dissecting microscope was used to determine extremes of the mycelium, and these points were marked on the tube with India ink. This distance was interpreted to represent linear growth of the organism in the soil. Reduction in linear growth caused by the fungicides was determined on a percentage basis by comparison with linear growth of mycelium in soil not containing fungicides.

Two isolates of *R. solani* were used. One was from a carnation grown in Maryland and the other from alfalfa from North Carolina.

As a result of preliminary *in vitro* tests, 3 water-soluble fungicides and an antibiotic were used as test materials. These were Puratized Agricultural Spray (7.5 per cent tris(2-hydroxyethyl) (phenylmercuri)ammonium lactate); Vancide 51 (30 per cent sodium dimethyldithiocarbamate and sodium derivative of 2-mercaptobenzothiazole); Di hane D-10 (96.7 per cent disodium ethylenebis[dithiocarbamate]); and Acti-dione (90 per cent 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide).

In all experiments the concentrations, based on active ingredients added to the soil, were Acti-dione, 10 ppm; Dithane D-10, 60 ppm; Puratized Agricultural Spray, 0.6 ppm; and Vancide 51, 60 ppm. Usually there was little change in soil pH as a result of addition of these materials.

As soon as data from 1 test were obtained, the experiment was repeated and the 2 sets of data combined. There were 3 replications of each treatment in all experiments.

EXPERIMENTS AND RESULTS.—A summarization of the data of these experiments on effect of soil factors on fungicidal activity is presented in Table 1. Values shown are the percentages of inhibition of growth of the isolate of *R. solani* from carnation. In most experiments behavior of the 2 isolates was very similar, but in a few instances they differed significantly.

In order to obtain some concept of the effect of soil texture on activity of fungicides in soil, the normally high clay content of Christiana soil was varied by addition of 30, 60, or 90 per cent sand. Each resulting soil texture was treated with each of the 4 fungicides and infested with *R. solani*. The percentage of inhibition of growth of 2 isolates of this pathogen increased significantly as the amount of sand increased. It is, perhaps, more logical to think of this effect as a decrease in fungicidal activity with an increase in clay and silt content of the soil.

Another soil factor affecting fungicidal activity was organic matter content. The type of organic matter present in soil was shown to be of significance, since addition of 1 per cent by weight of corn starch caused a reduction in the effect of Dithane D-10 and Puratized Agricultural Spray on both isolates. Starch reduced the activity of Vancide 51 only in the case of

TABLE 1.—*Percentage inhibition of R. solani by fungicides in soil in which several factors were varied*

Soil conditions used	Percentage inhibition by indicated fungicide of mycelial growth in soil				Linear growth (mm) in soil without fungicide
	Acti-dione 10 ppm	Dithane D-10 60 ppm	Puratized Agricultural Spray 0.6 ppm	Vancide 51 60 ppm	
30 per cent sand	43[a]	39	24	6	59
60 per cent sand	75	55	49	22	52
90 per cent sand	86	69	67	60	63
10.5 per cent moisture	54	71	58	24	64
15.0 per cent moisture	76	77	53	37	56
19.5 per cent moisture	84	83	54	44	50
21°C soil temperature	77	78	76	29	65
30°C soil temperature	85	79	55	39	93
Soil + fungicide	81	68	45	45	98
Soil + fungicide + 1% starch	85	25	28	38	69
Soil + fungicide + 1% casein	83	67	41	42	100
Soil + fungicide	77	78	51	48	89
Soil + fungicide + 1% clover hay	79	53	32	43	84
Soil + fungicide + 1% wheat straw	74	59	32	42	88
Soil + fungicide	84	79	46	35	77
Soil + fungicide + 1% clover hay	75	52	33	43	90
Soil + fungicide + 5% clover hay	64	37	22	40	98
pH 4.6	74	64	31	33	72
pH 6.1	50	85	67	87	97
pH 7.1	18	79	47	74	73

[a] Figures represent percentage reduction of linear growth of mycelium in comparison with linear growth in soil free of fungicides. LSD values at 5 and 1 per cent, respectively, are as follows: sand content, 6.8 and 9.3; moisture content, 24.5 and 33.3; temperature, 20.0 and 27.7; starch and casein, 11.8 and 16.0; hay and straw, 16.4 and 22.2; 1 per cent hay and 5 per cent hay, 9.9 and 13.4; and soil pH, 12.5 and 17.0. Corresponding LSD values for linear growth of mycelium in soil free of fungicides are the following: sand levels, 15.6 and 24.4; moisture levels, 6.3 and 9.9; temperature, 3.9 and 7.2; starch and casein, 17.8 and 27.9; hay and straw, 8.4 and 13.1; 1 per cent hay and 5 per cent hay, 10.6 and 16.6; and soil pH, 14.1 and 23.8.

the alfalfa isolate, and it had no significant effect on Acti-dione. It is of interest that 1 per cent starch in the soil caused a reduction in fungus growth even when fungicides were absent. In contrast to this effect of a carbohydrate, addition of a protein (casein) had no apparent effect on activity of any of the fungicides. Addition of raw plant material to soil also affected activity of fungicides. Clover hay and wheat straw were dried at room temperature (about 25°C), ground with a meat grinder, and passed through a 50-mesh screen. When either material was added to soil to the extent of 1 per cent by weight, they reduced activity of Dithane D-10 and Puratized Agricultural Spray but did not affect that of Vancide 51 or Acti-dione. To determine the effect of organic matter level, clover hay prepared as described was added to soil at the rates of 1 or 5 per cent by weight. Again the effect was most pronounced on Dithane D-10 and Puratized Agricultural Spray, for there was a highly significant decrease in fungicidal activity at the higher rate of hay. Activity of Acti-dione was decreased at the 5 per cent level, whereas activity of Vancide 51 was not affected.

Since the previous tests indicated that adsorptive power of soil might be of much importance in governing activity of soil fungicides, the effect of soil pH was investigated. Three soil pH values, 4.6, 6.1, and 7.1, were used. For these tests, the normal acidity of 4.6 was increased by adding 1N NaOH. Activity of Vancide 51, Dithane D-10, and Puratized Agricultural Spray increased as the pH value of the soil increased. In sharp contrast was the decrease in activity of Acti-dione. Apparently the maximum activity of Vancide 51, Dithane D-10, and Puratized Agricultural Spray was near pH 6.1, whereas for Acti-dione it was at a more acid reaction.

To determine the effect of soil moisture on fungicidal activity, sterile distilled water to the extent of 10.5, 15.0, or 19.5 per cent by weight was added to soil. Under conditions of these experiments, soil moisture level had very little effect on activity of soil fungicides. Likewise, the soil temperatures tested were of relatively little importance in this respect, since there was no significant difference in activity of the 4 fungicides at 21° and at 30°C.

Discussion.—In accord with previous work (1, 2, 6, 7, 14, 16) results of this investigation showed that soil texture affects activity of fungicides. Likewise, the effect of soil acidity as reported here was similar to that reported by others (4, 6, 7, 14, 16). This was especially true in the case of Acti-dione where an acid condition favors activity. The finding of Dunlap (4) that an increase in soil moisture did not affect activity of chemicals in the soil was supported by this investigation.

No published report was found on the effect of the type of organic matter in soil on fungicidal activity. It is apparent from the data reported herein that type and level of organic matter may have a pronounced effect on fungicidal action.

Results of this work are interpreted as indicating that the most important factor controlling activity of fungicides in the soil is adsorption. The effects on fungicidal activity of change in pH; in type, level, and kind of soil organic matter; and in the clay content of the soil can be interpreted as the direct effect of alteration in the adsorptive power of the soil. If this is true, any other factor that affects adsorption would affect activity of fungicides in soil.

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THE INFLUENCE OF SEED TREATMENT AND PLANTING RATE ON THE EMERGENCE AND YIELD OF SOYBEANS [1]

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SUMMARY

In a 4-year test to determine the effect of seed treatment and planting rate on the emergence and yield of soybeans in spaced-row and large drill-plot tests, results were dependent upon seed quality, planting rate, and, to some extent, environmental conditions prevailing at the time of and following planting.

Treatment of poor quality seed with Arasan or Spergon usually improved the emergence and in some instances significantly increased the yield.

Under conditions of accurate hand-spacing, treatment of good quality seed resulted in only slightly improved emergence and no significant effect on yield except at the lowest seeding rate (4-in. spacing). At this low seeding rate, however, yields were significantly lower than from untreated seed at 1- to 3-in. spacing intervals.

In plots seeded with a conventional grain drill, yields were reduced regardless of seed treatment when seeding rates were below a calculated average seed spacing of 1½ in. because of the lack of uniformity of spacing obtained. This precluded the possibility of securing maximum yields by combining seed treatment and significantly reduced seeding rates under standard field practice.

The results of these trials conducted under conditions both favorable and unfavorable for emergence and establishment of stand indicate that seed treatment may be of value when seed of poor quality is used or seeding is at a very low rate. Under standard seeding rates and with the use of reasonably good seed, however, no practical value of seed treatment has been demonstrated.

Results of chemical treatment of soybean seed by several workers have shown increased emergence and stand, but yields commonly have not been significantly affected (1, 4, 5, 6, 7, 8, 9, 12, 14). The studies reported here were initiated to determine the value of chemical treatment of soybean seed and particularly the possibility of obtaining maximum yield from significantly reduced planting rates by the aid of seed treatment. They involved an analysis of the interactions of seed quality, planting rate, and seed treatment in terms of emergence and yield.

MATERIALS AND METHODS.—*Spaced-row tests.*—Individual row tests were conducted in 1947, 1948, and 1949 using a split-plot, 4-replicate design in 1947 and 1948 and 8 replicates in 1949. The rows, 18 ft. long, were planted 40 in. apart. Seed was hand spaced at intervals ranging from ½ in. (approximately 120 lbs. of seed per acre) to 4 in. (15 lb. per acre) in the different tests. Stand counts were made after emergence was complete. The center 16 ft. of each row was harvested for yield.

Four seed lots each of Richland (lots 2, 3, 6, and 7) and Lincoln (lots 1, 4, 8, and 9) were obtained from farmers' seed storage bins. The variety Patoka (lot 5) was included to represent a large-seeded variety and Hawkeye (lot 10) to represent a badly weather-damaged seed lot. Only the Lincoln variety was planted in the 1948 and 1949 tests. Seed was treated with Arasan dust (50 per cent bis(dimethylthiocarbamoyl) disulfide) at 2 oz. per bushel in 1947 and 1948 and with Arasan or Spergon dust (96 per cent tetrachloro-*p*-benzoquinone) at the same rate in 1949.

Large drill-plot tests.—Large plot tests designed to be comparable to the ordinary farming practice were conducted in 1949 and 1950. Seed was treated with Arasan or Spergon at the rate of 2 oz. per bushel. The Lincoln and Hawkeye varieties were used in 1949 and Hawkeye only in 1950. The planting rates were calculated to give average spacings between seeds in the row of approximately 1½, 2½, and 3½ in. in 1949 and 1, 1½, 2, 2½, and 3 in. in 1950. This provided seeding rates of 53, 32, and 23 lb. per acre for Lincoln in 1949; 68, 41, and 29 lb. per acre for Hawkeye in 1949; and 82, 54, 46, 33, and 27 lb. per acre for Hawkeye in 1950. The seed was planted with a grain drill in a split-plot, 6-replicate design. Each plot consisted of 4 rows, 28 in. apart and 160 ft. long in 1949 and 70 ft. long in 1950. The center 2 rows were used in measuring stand and yield.

Weather and soil conditions.—The weather and soil conditions following planting were different each year. In 1947 a period of hot, wet weather followed planting and caused a hard crust to form on the soil surface. The 1948 test was planted in extremely dry soil, and germination was delayed for 10 days until a moderately heavy rain fell. The weather was warm and the emergence rapid following the rain. Planting of the spaced-row test and of the variety Hawkeye in the drill-plot test in 1949 was followed by ideal

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temperature and moisture for emergence. The variety Lincoln in the 1949 drill-plots was planted prior to a cold, wet period of approximately 2 weeks' duration. In 1950, heavy rain and cool weather followed planting. Water stood for several days in 1 small area of the plot, but all treatments were affected similarly.

EMERGENCE.—*Spaced-row tests.*—The data on the influence of seed treatment with Arasan and planting rate on emergence are summarized in Table 1. Highly significant differences were obtained among seed lots, spacings, and treatments in 1947. The rate of planting had a marked influence on the emergence. The differences between the emergence at the ½-in. (69 per cent) and the 2- and 4-in. spacing (61 and 59 per cent, respectively) were highly significant, and the mean difference of 2 per cent between the 2- and 4-in. spacing was significant. A highly significant over-all increase in emergence of 4 per cent resulted from seed treatment. Seed treatment did not markedly increase the emergence of 4 of the 5 higher-germinating lots, however, but did increase the emergence of 4 of the 5 lower-germinating seed lots. The effectiveness of seed treatment was influenced by the rate of planting. A highly significant increase in emergence was obtained as a result of seed treatment at the heavy seeding rate, whereas at the low seeding rate seed treatment did not increase the emergence significantly.

In 1948, highly significant differences in emergence were obtained among seed lots and treatments. The treatment times lot interaction was highly significant. The differences among spacings were not significant, as they had been in the 1947 test. An average increase of 16 per cent resulted from seed treatment, but the lots did not respond equally to treatment. Seed treatment resulted in an average increase in emergence of only 7 per cent in lot 1, the highest quality seed, but produced average increases of 16 and 24 per cent, respectively, in lots 2 and 3, the poorer quality seed. Highly significant differences in emergence between treated and untreated seed resulted at all spacings. The differences were nearly the same at all spacings, rather than decreasing at the lower seeding rates as occurred in 1947. Neither seed treatment nor rate of planting had a significant influence on emergence in 1949.

Large drill-plot tests.—The emergence data for the drill-plot tests, being similar in trend to that from spaced-row tests, are not presented. Seed treatment with Arasan and Spergon of both Lincoln and Hawkeye produced either highly significant or significant increases in emergence over untreated seed in the 1949 test. These differences were relatively small. Arasan treatment of both varieties produced an increase in emergence of approximately 4.5 per cent. Spergon

TABLE 1.—*Emergence of Arasan-treated and untreated soybean seed planted at different intervals in spaced-row tests, Lafayette, Indiana, 1947-1949*

Year and lot number	Variety	Percentage germination	Percentage emergence at indicated seed spacings											
			½ in.		1 in.		2 in.		3 in.		4 in.		Mean	
			Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
1947														
1	Lincoln	97	85	78			74	79			69	69	76	75
2	Richland	93	87	83			71	71			63	76*	74	77
3	Richland	87	75	72			65	61			68	57	69	63
4	Lincoln	80	72	66			57	54			49	58	59	59
5	Patoka	80	79	76			73	73			74	68	75	72
6	Richland	72	73	69			61	60			58	61	64	63
7	Richland	72	72	70			66*	54			63	57	67*	60
8	Lincoln	70	66*	54			57	54			57	53	60	54
9	Lincoln	59	58	48			52	40			48	42	53**	43
10	Hawkeye	49	54	44			50	41			39	37	48*	41
Mean for treatment			72**	66			63*	59			59	58	65**	61
Mean for spacing[a]			69				61				59			
1948														
1	Lincoln	90			78	75	85**	68	75	76	82*	73	80**	73
2	Lincoln	76			68**	54	68**	51	71**	54	68**	53	69**	53
3	Lincoln	65			36**	19	46**	19	53**	27	49**	23	46**	22
Mean for treatment					61**	49	66**	46	66**	52	66**	50	65**	49
Mean for spacing[b]					55		56		59		58			
1949														
1	Lincoln	95			82	88	88	91	86	83	89	87	86	87
Mean for spacing[b]					85		90		85		88			

* Significant difference at 5 per cent level between treated and untreated.
** Significant difference at 1 per cent level between treated and untreated.
[a] LSD between means for spacing at 5 per cent level, 2; at 1 per cent level, 3.
[b] Differences not significant.

treatment, on the other hand, resulted in an increase in emergence of 8.6 and 6.4 per cent for Hawkeye and Lincoln, respectively.

Small increases in emergence were obtained by seed treatment with both Arasan and Spergon in the 1950 drill-plot test, but only with Spergon was the emergence significantly greater than from the untreated check.

YIELD.—*Spaced-row tests.*—In the 1947 test, the differences among lots, spacings, and the spacing times lot and treatment times spacing interactions were highly significant (Table 2). The difference between treatments was not significant.

The mean difference in yield between the ½- and 2-in. spacings was not significant, but the yield at both of these spacings was significantly greater than at the 4-in. spacing.

Seed treatment at the ½-in. spacing resulted in a significant reduction in yield. At the 2-in. spacing, treated seed produced slightly greater yields than untreated seed, and at the 4-in. spacing it produced slightly less than untreated seed. Neither difference was statistically significant.

In 1948, highly significant yield differences existed among seed lots, spacings, and treatments. The treatment times lot interaction was highly significant, and the treatment times spacing interaction was significant.

An over-all average increase in yield of 5.3 bushels per acre resulted from use of treated seed. The highly significant treatment times lot interaction indicated, however, that the increase in yield as a result of seed treatment varied with the different seed lots. Seed treatment of lot 1 resulted in a significant average increase in yield of 3.2 bushels per acre over untreated seed. Seed treatment of lot 2 produced an average increase of 2.6 bushels per acre, which closely approached the 2.7 bushels per acre required for significance. On the other hand, seed treatment of lot 3 resulted in a highly significant average increase in yield over untreated seed of 10.2 bushels per acre.

When all 3 lots are considered together, highly significant increases of 5.8, 6.3, and 8.3 bushels per acre resulted from the seed treatment at the 2-, 3-, and 4-in. spacings, respectively, whereas an average increase of only 1.1 bushel per acre was attained from the seed treatment at the 1-in. spacing. The reason for these differences and the significant treatment times spacing interaction becomes apparent from a comparison of treatments within lots and spacings. Lots 1 and 2 reacted similarly to treatment at each spacing. A slight reduction in yield accompanied seed treatment at the 1-in. spacing, and an increase in yield was attained from seed treatment at the other 3 spacings. Only at the 4-in. spacing, however, was the difference between yield from treated and that

TABLE 2.—*Yield of Arasan-treated and untreated soybeans planted at different intervals in spaced-row tests, Lafayette, Indiana, 1947-1949*

Year and lot number	Variety	Percentage germination	Bushels per acre yield at indicated seed spacings											
			½ in.		1 in.		2 in.		3 in.		4 in.		Mean	
			Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
1947														
1	Lincoln	97	35.9	37.9			38.4	36.6			34.6	34.0	36.3	36.2
2	Richland	93	28.9	28.7			31.0	29.2			29.8	29.2	29.9	29.0
3	Richland	87	31.9	33.4			31.6	30.8			28.0	31.2	30.5	31.8
4	Lincoln	80	36.1	37.4			35.4	34.2			32.0	32.4	34.5	34.7
5	Patoka	80	33.9	35.6			38.2	37.1			35.8	34.3	36.0	35.7
6	Richland	72	31.2	32.8			30.9	29.0			26.8	30.4	29.6	30.7
7	Richland	72	31.3	32.6			31.5	32.1			27.9	29.2	30.2	31.3
8	Lincoln	70	40.9	42.0			39.0	37.0			35.5	33.4	38.5	37.4
9	Lincoln	59	37.9	40.0			37.6	37.0			32.0	34.7	35.9	37.2
10	Hawkeye	49	34.0	34.2			32.2	34.8			28.0	28.0	31.4	32.3
Mean for treatment			34.2	35.4*			34.6	33.8			31.0	31.7	33.3	33.6
Mean for spacing[a]			34.8				34.2				31.4			
1948														
1	Lincoln	90			42.7	43.3	38.3	36.6	43.0	37.7	41.3*	34.8	41.3*	38.1
2	Lincoln	76			40.7	42.2	39.4	35.8	41.5	38.8	38.4*	32.6	40.0	37.4
3	Lincoln	65			44.1*	38.7	39.8**	27.7	38.4**	27.7	36.2**	23.4	39.6**	29.4
Mean for treatment					42.5	41.4	39.2**	33.4	41.0**	34.7	38.6**	30.3	40.3	35.0
Mean for spacing[b]					41.9		36.3		37.9		34.3			
1949														
1	Lincoln	95			30.4	29.9	31.8	30.9	29.1	29.6	28.5	27.6	30.0	29.5
Mean for spacing[c]					30.2		31.4		29.4		28.1			

* Significant difference at 5 per cent level between treated and untreated.
** Significant difference at 1 per cent level between treated and untreated.
[a] LSD between means for spacing at 5 per cent level, 0.8 bushel; at 1 per cent level, 1.0 bushel.
[b] LSD between means for spacing at 5 per cent level, 2.0 bushels; at 1 per cent level, 2.7 bushels.
[c] LSD between means for spacing at 5 per cent level, 1.4 bushels; at 1 per cent level, 2.0 bushels.

from untreated seed statistically significant. In lot 3, treated seed produced a significant increase in yield over untreated seed even at the 1-in. spacing and a highly significant increase at the other 3 spacings.

In the 1949 test, there were no significant differences in yield among chemical treatments, but significant differences in yield among spacings were obtained.

Large drill-plot tests.—There were no significant differences between yields from treated and untreated seed in either year, but significant differences in yields among rates of planting occurred in both years (Table 3). The highest yield in 1949 was obtained at the heaviest planting rate (1½-in. spacing), and each reduction in the seeding rate resulted in a marked reduction in yield. In 1950, the highest yield again was produced at the 1½-in. spacing, but it was not significantly larger than the yield at the 1-in. interval. Spacing intervals between seeds greater than 1½ in. resulted in sharply reduced yields.

Discussion.—The yearly differences between the comparative emergence of treated and untreated seed of similar quality indicate that environmental conditions at the time of and following planting influence the effect of seed treatment. The greatest increases in emergence were obtained in 1948 when the soil was so dry that germination was delayed 10 days. Under this condition, treatment increased emergence of both good and poor quality seed; however, the response of the poor quality seed was the greater. There was no significant difference between the emergence from treated and untreated seed in the spaced-row test conducted in 1949 when conditions were ideal for emergence. In the drilled plots in the same year, the total emergence of the variety Lincoln planted prior to a prolonged cold, wet period was considerably lower than the emergence of the variety Hawkeye, which had very favorable conditions for emergence. The percentage increases in emergence as a result of seed treatment, however, were no greater in the Lincoln plot under conditions generally considered unfavorable for emergence than they were in the Hawkeye plot under conditions considered ideal for soybean emergence.

Rate of planting influenced the effect of seed treatment on emergence in 1947, a year in which a hard crust formed on the soil surface after planting, and in 1950, when a light surface crust formed. The collective growing force of the seedlings enabled them to break through a relatively hard surface crust. This accounts for the greater percentage of emergence at the heavier planting rates and may explain the greater effectiveness of seed treatment at the heavier seeding rates. Emergence was slightly more rapid at the heavier seeding rates where the growing force was greater. This may have enabled a larger percentage of the weak seedlings, the ones benefited most by seed treatment, to emerge than could do so at lower seeding rates where lesser growing force was exerted and emergence was slower.

Yield is the most important consideration in determining the beneficial effect of seed treatment. In a number of seed-treatment studies (2, 3, 10, 11, 13, 16), only emergence data have been reported. In the present studies, seed treatment at times produced an increase in emergence, but these increases seldom were accompanied by an increase in yield. When the planting rate was at the commonly recommended rate of 45–60 lb. per acre (1–1½ in. between seeds), an increased yield as a result of seed treatment occurred only in 1948 and with the poorest lot of seed. In fact, treatment of good-quality seed at this rate of seeding occasionally resulted in a slight but nonsignificant reduction in yield. Apparently an adequate stand was obtained without seed treatment.

A significant difference was found between the yield from treated and untreated good-quality seed when the planting rate was reduced to approximately 15 lb. per acre (4 in. between seeds). The yield from the treated seed at this greatly reduced seeding rate,

Table 3.—*Yield of soybeans in large drill-plot tests in which treated and untreated seed was planted at various spacings*

Treatment	Bushels per acre yield at indicated seed spacings						Mean for treatment
	1 in.	1½ in.	2 in.	2½ in.	3 in.	3½ in.	
			1949 (Lincoln variety; 95 per cent germination)				
Arasan		30.4		25.8		22.9	26.4
Spergon		29.4		27.0		23.9	26.8
Untreated		29.4		27.3		22.2	26.3
Mean for spacing[a]		29.7		26.7		23.0	
			1949 (Hawkeye variety; 93 per cent germination)				
Arasan		33.3		30.2		25.2	29.6
Spergon		33.1		30.9		27.1	30.4
Untreated		32.0		29.8		26.4	29.4
Mean for spacing[b]		32.8		30.3		26.2	
			1950 (Hawkeye variety; 85 per cent germination)				
Arasan	28.8	29.0	25.6	23.3	22.5		25.8
Spergon	29.1	28.1	26.0	21.5	22.4		25.4
Untreated	26.8	28.3	26.8	22.1	25.2		25.8
Mean for spacing[c]	28.2	28.4	26.1	22.3	23.4		

[a] LSD between means for spacing at 1 per cent level, 1.7 bushels.
[b] LSD between means for spacing at 1 per cent level, 1.4 bushels.
[c] LSD between means for spacing at 5 per cent level, 3.2 bushels; at 1 per cent level, 4.3 bushels.

however, generally was significantly lower than that produced from untreated seed planted at rates between 30 and 60 lb. per acre.

The highest yield in the spaced-row plots sometimes was produced from seed planted 2 in. apart, but the drill-plot tests demonstrated that with the conventional planter it was not possible to regulate the seed-drop accurately enough to obtain a uniform stand at planting rates below an average of 1 seed to every 1 to 1½ in. in the row. Consequently, skips of 4–8 in. between plants often resulted when the seed was planted at reduced rates calculated to give an average spacing of 2 in. or greater between seeds, and at these rates the yields were consistently lower. This is in agreement with the finding of Probst (15) that the highest yield was usually obtained when the actual spacing between plants was 2–3 in. and that there was no appreciable drop in yield until the spacing between plants was less than 1 in. or greater than 4 in.

The fact that the actual stand can vary so greatly, from 1 to 4 in. between plants in the row, without significantly affecting yield explains to some extent why increases in emergence as a result of seed treatment are so rarely accompanied by increases in yield. It also indicates that the percentage increase in emergence does not reflect the beneficial effect of seed treatment as measured by yield unless there is such a poor or nonuniform stand that there are numerous skips greater than 4 in. between plants.

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METHODS OF INOCULATION FOR WILDFIRE OF SOYBEAN AND THE EFFECT OF BACTERIAL PUSTULE ON WILDFIRE DEVELOPMENT[1]

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SUMMARY

A comparison of 6 different methods of inoculation showed that the wildfire disease of soybeans developed best when associated with the bacterial pustule disease. This was true whether the wildfire inoculum was sprayed over pustule-affected leaves, or whether *Pseudomonas tabaci* and *Xanthomonas phaseoli* var. *sojensis* were introduced simultaneously into the leaf by watersoaking. Previous infection with the bacterial blight organism *Pseudomonas glycinea* also favored wildfire infection. Watersoaking the healthy leaf with wildfire inoculum alone resulted in erratic infection. Wounding was an ineffective method of inducing infection. Healthy leaves sprayed lightly with inoculum without watersoaking developed no infection.

In 1946 and 1947, a group of soybean varieties was inoculated with the wildfire organism, *Pseudomonas tabaci* (Wolf & Foster) F. L. Stevens, at Urbana, Illinois. Although 2 modes of entry were provided for the organism, namely, abundant leaf injury in the form of insect punctures and driving the inoculum directly into the stomata with a power sprayer, only traces of infection resulted. Field observations during this same period showed that each natural outbreak of wildfire was associated with the bacterial pustule disease caused by *Xanthomonas phaseoli* var. *sojensis* (Hedges) Starr & Burk., with a pustule usually visible at the center of a wildfire lesion. Graham[3] found that bacterial pustule on soybean leaves facilitated wildfire development and considered the pustule as the usual port of entry for the wildfire organism in the field. Since bacterial blight caused by *Pseudomonas glycinea* Coerper usually precedes wildfire in the field, it seemed possible that blight might be similarly associated with the epiphytology of wildfire. This paper reports the results of experiments on the relationship between bacterial pustule and wildfire and on the relative effectiveness of several methods of inoculation.

Preliminary field experiments in 1948 indicated that infection with the wildfire organism occurred more readily on leaves with the pustule disease than on healthy leaves. The results were unsatisfactory, however, because natural infection by the pustule organism could not be controlled. Subsequent experiments were performed in the greenhouse, where pustule-free controls could be maintained.

METHODS.—Cultures of *Pseudomonas tabaci* were grown 4–5 days in Wernham's buffered potato-dextrose broth, then centrifuged and centrifuge-washed 3 times in sterile distilled water to remove the exotoxin. This latter step is necessary because the toxin can induce typical halos in the absence of *Ps. tabaci*.[4]

The washed bacteria were then resuspended in sterile distilled water. The suspension was standardized to approximately 8×10^8 cells per ml and diluted 1-100 for inoculum. The same procedure was followed in preparing inoculum for the pustule and blight organisms. Isolates of *Ps. tabaci* from soybeans were used in most of the tests, but 2 tobacco isolates were also included in 2 experiments for comparison.

Comparisons were made of the following methods of inoculation: 1) pustule-affected leaves sprayed lightly with the wildfire inoculum; 2) pustule-affected leaves watersoaked with the wildfire inoculum; 3) healthy leaves watersoaked with inoculum containing both wildfire and pustule organisms; 4) leaves with bacterial blight sprayed lightly with the wildfire inoculm; 5) healthy leaves watersoaked with wildfire inoculum only; and 6) leaves wounded by needle-puncture and sprayed with the wildfire inoculum. As a control, healthy uninjured leaves were sprayed lightly without watersoaking with wildfire inoculum.

The "half-leaf" technique was used to compare the different methods of inoculation. Treatments 1 and 5 were compared on opposite halves of the same leaves, and used as standards for comparison with the other methods. Each pair of treatments was replicated on the 2 topmost fully developed leaves of 6 Lincoln soybean plants.

RESULTS.—Table 1 shows results typical of the ranges of infection obtained through 4 experiments with the various methods of inoculation. Paired treatments are connected by broken lines. It is evident that pustules provided portals of entry for the wildfire organism and that bacterial blight lesions functioned likewise (treatments 1 and 4), since the controls showed no infection. Moreover, all inoculations involving the pustule and wildfire organisms in combination resulted in more consistent infection with the latter than did those involving *Ps. tabaci* alone. This confirms Graham's results[3].

Judging by the maximum infection induced, infection

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[3] Graham, J. H. 1953. Cultural and epiphytotic relationships of three bacterial pathogens of soybeans. Phytopathology 43: 193-194.

[4] Clayton, E. E. 1934. Toxin produced by bacterium tabacum and its relation to host range. Jour. Agr. Res. 48: 411-426.

TABLE 1.—*Infection of Lincoln soybean plants by Ps. tabaci following inoculation by 6 different methods*

Isolate[a]	Percentage infection for the following methods of inoculation[b]						
	1	2	3	4	5	6	Control
S1 and S2 mixed	75			11			
	92			8			
	78				0		
	44					0	0
	15			67			
		47			0		
	70					0	
S1		58			38		0
	33				2		
			61		7		
	33			78			
S2			78		35		0
T1			43		4		
T2			61		14		

[a] S1, S2 = soybean isolates; T1, T2 = tobacco isolates.

[b] 1 = Wildfire inoculum sprayed lightly over leaves showing pustules; 2 = leaves infected with pustule organism watersoaked with wildfire inoculum; 3 = healthy leaves watersoaked with inoculum containing both pustule and wildfire organisms; 4 = wildfire inoculum sprayed lightly over leaves showing blight lesions; 5 = healthy leaves watersoaked with wildfire inoculum; 6 = wounded leaves sprayed lightly with wildfire inoculum. Control = wildfire inoculum sprayed lightly over healthy, uninjured leaves. Figures connected by broken lines represent data from opposite half leaves. Percentage infection equals percentage of total inoculations developing halos.

by the blight organism also seemed to favor infection by *Ps. tabaci* (treatment 4). On plants given this treatment, a blight lesion lost its identity when a wildfire lesion developed around it. Wounding was ineffective on soybeans, although it is usually successful on tobacco. The control plants developed no symptoms. In relation to the pustule organism, the time elapsing between inoculation and appearance of halos was the same whether wildfire inoculations were made on pustule-affected leaves or whether the pustule and wildfire organisms were introduced simultaneously.

A further test was made to determine the effect of the pustule organism on the multiplication of *Ps. tabaci* in the leaves of tobacco and soybean. The topmost fully developed leaf on 3 plants of each species was inoculated as follows: the left half of each leaf was watersoaked with inoculum containing a mixture of the pustule and wildfire organisms, while the right half was watersoaked with inoculum made with *Ps. tabaci* alone. The same isolate of the wildfire organism, originally taken from soybean, was used in both cases. Four days after inoculation, 6 samples (2 from each half-leaf) were taken with a 4-mm cork borer, ground thoroughly in 10 ml of sterilized water, diluted appropriately, and plated on potato-dextrose agar. When tobacco leaves were used, colony counts (*Ps. tabaci*) per 4-mm leaf-sample were 2300 for *Ps. tabaci* alone and 28,000 when it was associated with the pustule organism. When soybean leaves were used, the counts were 36,000 and 201,000, respectively. The experiment was repeated with similar results. On tobacco, there was no evidence of pathological damage attributable to the pustule organism and no increase in the number of wildfire lesions on leaves inoculated with both organisms. On soybeans, there was a 2- to 4-fold increase in the number of halos on leaves inoculated with both organisms, compared with leaves inoculated with *Ps. tabaci* alone.

DISCUSSION.—The results of these experiments offer an explanation for the frequent failure of experimental inoculations on soybeans with *Ps. tabaci*. Clayton's[5] inoculations were made in the absence of other soybean leaf diseases, which probably accounts for the low level of infection he obtained in field trials. Graham[3] found that previous infection by the pustule organism favored the development of wildfire. He contended that the pustule lesion served as the usual point of entry for *Ps. tabaci* and theorized that the killing of host cells by the pustule organism is a necessary prerequisite for the development of the wildfire bacteria. While the data in Table 1 (treatment 1) support the idea that field infections take place through pustule lesions, the results of treatments 2, 3, and 5 indicate a more fundamental relationship between the 2 organisms, wherein the pustule organism creates an environment favorable for the establishment and multiplication of the wildfire organism. Entry of the wildfire organism is not a factor in any of these 3 treatments, since the bacteria were introduced into the leaf tissues by water-soaking. The high percentage of wildfire lesions obtained when the pustule and wildfire organisms were introduced simultaneously into the leaf indicates that previous pathological damage by the pustule organism is not necessary for infection by *Ps. tabaci*. Moreover, if cellular damage were the predisposing factor necessary for the establishment of *Ps. tabaci*, one might logically expect wildfire symptoms to develop sooner on leaves previously affected by pustule than on healthy leaves inoculated with both organisms simultaneously. There was no such time differential in these experiments. *Ps. tabaci* produced the typical exotoxin and multiplied within the soybean leaf (treatment 5) in the absence of the pustule organism, showing that in greenhouse plants its pathogenicity does not depend entirely on association with other organisms. Limited evidence that *Ps. tabaci* multiplied more rapidly in tobacco leaves in association with the pustule organism than it did alone further indicates that the beneficial effect of the pustule organism is something other than pathological damage, since the pustule organism causes no pathological changes in tobacco. Although the nature of this relationship remains in doubt, it is evident that *Ps. tabaci*, which is not a virulent primary parasite on soybeans, can cause serious damage when associated with the pustule organism. There is some evidence that the bacterial blight organism may bear a relationship to *Ps. tabaci* similar

[5] Clayton, E. E. 1950. Wildfire disease of tobacco and soybeans. U. S. Dept. Agr. Pl. Dis. Rptr. 34: 141-142.

to that of the pustule organism. Further evidence on this point is difficult to obtain for 2 reasons: 1) both *Ps. tabaci* and *Ps. glycinea* produce white colonies on agar and hence are indistinguishable; and 2) the blight lesion loses its identity when surrounded by a wildfire lesion and cannot be recognized in the field.

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RESISTANCE IN PEPPER TO MELOIDOGYNE INCOGNITA ACRITA [1]

W. W. Hare

SUMMARY

A collection of 162 varieties or strains of pepper was tested for reaction to the root-knot nematode, *Meloidogyne incognita acrita*. Tests were conducted in infested soil in a greenhouse bench. No variety was found to be immune. Four varieties were grouped as highly resistant, 14 as moderately resistant, and 135 as susceptible. Nine were not rated. In large scale tests, commercial bell varieties did not differ in degree of susceptibility. Apparently resistant individuals in susceptible lots were demonstrated to be primarily escapes.

During the late summer of 1949 a group of pepper varieties (*Capsicum frutescens* L.) growing in a greenhouse bench were removed to determine the cause of very poor growth. The plants were heavily attacked by root-knot nematodes, and there appeared to be considerable varietal differences. A large number of pepper varieties or strains were at hand and they were compared in reaction to that culture of nematodes. This paper presents the results obtained. An earlier brief report (5) has been made.

Only 1 other description of resistance in pepper to root-knot nematodes has been found. Martin (8), in South Carolina, made selections in Cayenne pepper types for root-knot resistance, and the nematode-resistant variety Carolina Hot was released in 1954. The species of root-knot nematodes used in testing for resistance were not identified. From Martin's program, seed of 3 strains[2] were received for comparison to local ones in 1950. In nonreplicated rows, 1 strain had a lower root-knot index than the resistant check used, 1 was intermediate, and 1 was susceptible.

Resistance and tolerance to root-knot nematodes has been reported for numerous other plant species and for horticultural varieties. Tyler (11) in 1941 summarized those reports to approximately that date with no mention of pepper except for *Piper nigrum* L. One of the first descriptions of resistance in a cultivated species containing susceptible varieties was that of Iron cowpea by Webber and Orton (12) in South Carolina in 1902. Weimer and Harter (13) found some sweet potato varieties highly resistant in California in 1925. Among other reports were those of Isbell (7) on garden beans in 1931, and Bailey (1) on tomatoes in 1941.

MATERIALS AND METHODS. — *The nematode.* — The author was not aware of the separation of the root-knot nematode into several species (3) at the time the work reported here was carried out. Identification of the nematode involved was made by Nematologist A. L. Taylor[3], from material grown later in soil taken from the test bed, as the sub-species *Meloidogyne incognita acrita* Chitwood. He directed confirmatory tests on certain hosts and comparisons with a pure culture.

Growing and inoculation.—The techniques of Barrons (2) and Bailey (1) were adapted for growing the plants in infested soil. The galled roots of the original pepper were removed, chopped, and mixed into a layer of soil (Ruston sandy loam) about 5 in. deep in a transite bench. Three crops of Extra Early Blackeye cowpeas were grown to the 3-leaf stage. Each crop was removed, and the galled roots of the plants chopped up and mixed into the soil in the bench. Peppers were grown after the third crop of cowpeas, and after each crop of peppers the procedure was repeated—galled roots were chopped up and mixed with the soil. A mixed fertilizer (5–10–5) was added as needed. The bench was 48 in. × 40 ft. in

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dimension. Rows 6 in. apart were run across the bench and divided into groups of 4 rows 12 in. long in the first trial and into groups of 3 rows 15 in. long in subsequent trials. Twenty seed from the same source were planted per row in the first trial and 30 per row in subsequent trials. Final plant stands were variable, depending mostly on the vigor of the seed stock, but influenced somewhat by the replicate. For example, in trial 3, an average of 16.7 plants per lot, or 55.7 per cent of the seed planted, were classified, and the number varied from 14.5 to 19.2 in the different replicates.

Rating.—Plants were removed from the bed as soon as feasible after susceptible checks had shown well-developed galling and stunting. The growing period ran from 58 days in the second trial to 82 days in the third. Roots of the plants were washed, examined, and placed individually into arbitrary classes based on an estimation of the number and size of galls and the amount of root destruction. Classification was based on modifications of Smith and Taylor's (9) and Barron's (2) descriptions. The classes were defined as follows: 0) healthy—those plants with no signs of galls or other appreciable damage to the roots, and no marked stunting of plants; 1) slight—any definite galls, stubbing or darkening of the roots, or plant stunting evident, up to $\frac{1}{3}$ of the roots galled if plant was otherwise healthy; 2) moderate—plants with $\frac{1}{3}$ to $\frac{2}{3}$ of the roots galled, those with considerable stubbing of roots and stunting of plants, and those with large galls if roots were extensive; 3) severe—plants with over $\frac{2}{3}$ of the roots galled, those with few roots and 1 or more large galls, and those with no galls but plants very stunted and roots few and stubbed; and 4) dead. Although it was obvious that the nematodes were killing some of the plants, it was difficult to distinguish between killing by nematodes and damping-off. For this reason, the principal criterion used in placing plants in the dead class was dying of a stunted plant from the top down. Plants recognized as damped-off were discarded. Representatives of the first 4 classes are shown in Figure 1.

After the plants were classified, a root-knot index was calculated for each lot. Values were assigned classes as follows: healthy, 0; slight, 25; moderate, 50; severe, 75; and dead, 100. The number of plants in each class was multiplied by the class value, the totals added, and the sum divided by the total number of plants. An analysis of variance was made on the data from each of the 4 trials. For the purpose of analysis, the root-knot indices were transformed to angles corresponding to percentages (10).

Results.—*Variety trials.*—Varieties or strains were terms used to include commercial varieties of pepper and numbered accessions. Seed lots of a commercial variety from different sources were handled as separate entries. In the case of numbered accessions, no 2 that appeared to be the same were retained. In some instances, 2 or more distinct types were found within a single seed lot; these were retained under the same number with letter designations for identification.

Space prohibited testing all of the variety collection at 1 time; therefore, it was divided and 2 trials were made. In the first trial, 4 replicates of randomized complete blocks were planted December 2, 1949, and classified February 14-17, 1950. Attack by nematodes was not uniform throughout the bench. This was particularly true in replicate I at 1 end of the bench, and that replicate was omitted in the analysis. The average indices from replicates II, III, and IV for 76 varieties are shown in Table 1.

Five varieties were omitted from the table because of an insufficient number of plants. There were no discontinuous classes but rather a complete range from highly resistant to very susceptible with no variety entirely free from galls. The use of Duncan's multiple range test (4), however, showed that the drop in resistance was rather rapid and that most varieties were in the susceptible group. Santanka xS was significantly better at the 5 per cent level than all but the next 4 ranking varieties. On the other hand Red Chili, the eighth ranking variety, was not highly significantly better than the 73rd, 4786 Brazil.

The other half of the variety collection was grown after the soil was worked thoroughly and mixed with the galled roots from the previous crop. Planting was on February 28, 1950, and the plants were rated April 26 and 27, 1950. There were 81 varieties or strains arranged in a lattice design with 3 replications. Infection was at a much higher level and more uniform than in the first trial (Table 2). Four were omitted from the table because of insufficient plants. Again there was a rapid rise in the index level, with 405B Mexico better at the 5 per cent level than any except the next 7 varieties.

For a more direct comparison of those showing resistance and susceptibility in the 2 different trials, selections of varieties or strains were made from both levels. The 13 with the lowest indices and 3 with high indices were taken from each group. Four commercial varieties were included as susceptible for a total of 36 varieties, 26 resistant and 10 susceptible. These were arranged in a lattice design with 6 replications and planted in the bench on May 3, 1950. Final notes were taken July 24 and 25, 1950. The same test was repeated, planting on July 27 and 28, with the final notes taken September 26 and 27, 1950. Infection was very uniform for both trials and at a high level. Growing conditions were better, however, and there was no killing of the plants in the third trial and very little killing in the fourth (Table 3).

As in previous trials, there was a range from high resistance to complete susceptibility, with a rapid rise in the root-knot index after the first few varieties. The use of Duncan's test showed that there was a fairly clear-cut break after the first 4 varieties, 405B Mexico, 526 "Merceh 21", Santanka xS, and 4575P4, and a less definite break between the 18th (Red Chili) and 19th (46136) varieties. The first 4 varieties, with indices averaging less than slight, were grouped as highly resistant. Entries from the fifth variety, 405 Mexico, through the 18th, Red Chili, were considered

FIG. 1–2.—Fig. 1. From left to right: representatives of healthy, slight, moderate, and severe classes of root-knot on pepper. The nematode was *M. incognita acrita*.—Fig. 2. From left to right: Oakview Wonder, Red Chili, and 405B-Mexico pepper plants attacked by *M. incognita acrita*. These varieties represent susceptible, moderately resistant, and highly resistant groups, respectively.

TABLE 1.—*Angles of root-knot indices of pepper varieties or strains grown for 75 days in soil infested with M. incognita acrita*

Variety or strain and origin[a]	Root-knot index[b]	Variety or strain and origin[a]	Root-knot index[b]
45107 Santanka xS (a)	5.8	4699B Brazil (a)	44.7
P.I. 135873 Baluchistan (b)	10.6	4697 Brazil (a)	45.3
4646 Bird's Eye (a)	16.3	Blanco del Pai (g)	45.8
P.I. 135824 Afghanistan (b)	24.4	14 (g)	45.9
P.I. 155299 Arabia (b)	29.2	Hungarian Yellow Wax (d)	46.0
Red Chili Hot (c)	31.1	P.I. 135822—Afghanistan (b)	46.0
4656C Hungarian Yellow Wax (a)	31.3	4658 Peru (a)	46.0
Red Chili (d)	33.2	4694 Brazil (a)	46.2
4652C Louisiana Sport (a)	34.3	4656b Red Cherry (a)	46.3
Manatee Wonder (c)	34.8	Pepper Bell (e)	47.0
4651 TBM Resistant (a)	35.6	Muro (g)	48.0
4695 Brazil (a)	35.8	4656aB Yucatan (a)	48.1
Oakview Wonder (d)	36.4	4617 Yatsuffa (a)	48.5
P.I. 155299B-Arabia (b)	36.9	4651B TBM Resistant (a)	48.8
Anaheim (d)	37.1	4782 Paprika (a)	50.2
Imp. Cal. Wonder (e)	38.2	World Beater (c)	50.6
Merrimack Wonder (f)	38.2	4657 Peru (a)	51.8
4654 Cayenne (a)	38.4	Pimiento (d)	52.2
4699 Brazil (a)	38.5	4656a Yucatan (a)	52.4
46100 Brazil (a)	38.7	4616a Hontanka (a)	53.7
Improved Ruby Giant (e)	38.9	1701(6) 26B (i)	54.1
Long Red Cayenne (d)	39.3	4674 Peru (a)	54.6
4781 Bird's Eye (a)	39.3	Mexican Chili (d)	54.8
Ruby King (h)	40.1	4698 Brazil (a)	54.8
1701 (2) 32 (i)	40.4	4664 Peru (a)	55.1
P.I. 135827 Afghanistan (b)	40.9	4648 California Wonder (a)	55.5
4652cB Louisiana Sport (a)	41.9	4673 Peru (a)	55.6
P.I. 135826 Afghanistan (b)	42.2	4660 Peru (a)	57.4
P.I. 135874 Baluchistan (b)	42.4	4654B Cayenne (a)	58.6
St. Jordi (g)	42.6	4661 Peru (a)	59.4
17 (g)	43.6	Florida Giant (e)	60.2
Sunnybrook (d)	43.7	4785 Brazil (a)	64.2
Taro (g)	43.7	4682 Brazil (a)	68.7
Cal. Wonder x Ruby King (h)	44.1	4677 Peru (a)	69.7
Blight Resistant World Beater (e)	44.4	4786 Brazil (a)	69.7
California Wonder (d)	44.5	4795 Peru (a)	72.2
4617B Yatsuffa (a)	44.5	4788 Peru (a)	77.5
4647 Floral Gem (a)	44.7	4790 Peru (a)	86.9

LSD 5 per cent = 22.3
LSD 1 per cent = 29.4

[a] Most of the pepper was collected by Dr. H. H. Foster. Names and/or numbers are given as received by him. The small letter in parenthesis indicates one of the following sources: (a) Dr. W. H. Greenleaf, Georgia Experiment Station, Experiment, Ga.; (b) Dr. W. S. Porte, U.S.D.A., Beltsville, Md.; (c) Assoc. Seed Growers, Inc., New Haven, Conn.; (d) Ferry-Morse Seed Co., Detroit, Mich.; (e) Kilgore Seed Co., Plant City, Fla.; (f) Dr. J. R. Hepler, University of New Hampshire, Durham, N. H.; (g) Dr. K. A. Bartlett, Mayaguez Federal Station, P. R.; (h) Local sources; and (i) Dr. F. O. Holmes, The Rockefeller Institute for Medical Research, Princeton, N. J.

[b] The number of plants in each root-knot class was mulitiplied by the class value (healthy, 0; slight, 25; moderate, 50; severe, 75; and dead, 100), the totals added, and the sum divided by the total number of plants to obtain the root-knot index for each entry.

moderately resistant with varying degrees of such resistance. The remaining varieties were considered susceptible. Representatives from these groups are shown in Figure 2.

Five of the 26 varieties selected from the first 2 trials as probably possessing some resistance were among the most susceptible varieties in the more critical tests. Four of these came from the first trial, where infection was not uniform. One of the varieties selected as susceptible, Korea-3, 1947, rated 14th and moderately resistant in the more critical trials. Its rating in trial 2 was based on 23 of 29, 26 of 27, and 25 of 28 dead of total plants in the respective replicates. In all probability, damping-off was confused with killing by the nematodes. This evaluation indicated that errors in placement in the earlier trials were primarily due to escapes that were rated as resistant. In the variety collection as a whole, there were 162 varieties or strains grouped as follows: 4 highly resistant, 14 moderately resistant, 135 susceptible, and 9 not included in the comparisons. The susceptible group probably included a few that could be rated moderately resistant in extensive tests.

Large-scale tests of susceptible varieties.—Some of the standard commercial varieties used in Mississippi were tested in large numbers. All such varieties had

TABLE 2.— *Angles of root-knot indices of pepper varieties or strains grown for 58 days in soil infested with the root-knot nematode, M. incognita acrita*

Variety or strain and origin[a]	Root-knot index[b]	Variety or strain and origin[a]	Root-knot index[b]
405B Mexico (a)	35.9	46109 Argentina (c)	63.3
526 "Merceh 21" (a)	41.9	Korea 1-1947 (f)	63.6
Anaheim Chile (b)	44.7	Truheart (g)	63.8
AC 390-1 (a)	45.9	P.I. 143886 Mexico (a)	63.8
46116 Mexico (c)	46.9	P.I. 157062 (a)	63.9
P.I. 141927 Manchuria (a)	50.6	Hantalca 2 Japan (a)	64.1
46136 (c)	51.5	P.I. 142828 Iran (a)	64.2
No. 72 (d)	52.1	No. 115 (d)	64.3
46111 Argentina (c)	53.0	46138 (c)	64.6
46107 Argentina (c)	53.2	688 China 1947 (a)	64.7
4575P4 (e)	53.8	4509 (e)	65.1
Italian Pickling (b)	53.9	Korea 2—1947 (f)	65.1
405 Mexico (a)	54.3	339 Africa (a)	65.7
46143 (c)	54.9	P.I. 141928 Manchuria (a)	66.0
46102 Brazil (c)	55.3	Hantalca 1 Japan (a)	66.1
409 Mexico (a)	55.6	P-20 (h)	66.4
46126 Puerto Rico (c)	56.2	P.I. 129332 Brazil (a)	67.2
45107B Santanka xS (c)	57.2	46106 Argentina (c)	67.4
46124 Puerto Rico (c)	57.7	P.I. 142841 Iran (a)	67.4
46142 (c)	58.4	4795B Peru (c)	67.6
P.I. 142829 (a)	58.6	Banana Sweet Pickling (j)	67.9
4530PI (e)	59.0	P.I. 135825 Afghanistan (a)	68.2
47107 (c)	59.4	47102 (c)	69.0
Red Sport (g)	59.4	Ruby King selection (g)	69.2
393-2 Mexico (a)	60.6	No. 73 (d)	71.4
46119 Mexico (c)	60.7	HES 75 (i)	71.7
HES 97 (i)	60.8	Cal. Wonder (j)	72.5
P.I. 140375 Iran (a)	61.0	46125 Puerto Rico (c)	72.9
47108 Santanka xS (c)	61.4	750 Hungary (a)	73.2
Harris Early Giant (b)	61.6	46132 Chile (c)	73.8
Pimiento (g)	61.7	47101 (c)	73.8
Waialua (i)	61.7	HES 70 (i)	73.9
HES 77 (i)	61.8	Chili Pequin Mexico (a)	75.1
687 Mexican Chile (a)	62.1	P97-10-1 (a)	76.1
Truheart (g)	62.2	46101 Brazil (c)	77.3
No. 71 (d)	62.4	Ozark Giant (b)	79.7
Floral Gem (b)	62.6	California Wonder (g)	79.7
Cal. Wonder Special (j)	62.6	Korea 3-1947 (f)	80.3
46100 Brazil	63.2		

LSD 5 per cent = 14.4
LSD 1 per cent = 19.0

[a] Seed sources are indicated by small letters in parenthesis as follows: (a) Dr. P. G. Smith, University of California, Davis, Calif.; (b) Assoc. Seed Growers, Inc., New Haven, Conn.; (c) Dr. W. H. Greenleaf, Georgia Experiment Station, Experiment, Ga.; (d) Dr. E. H. Casseres, Turrialba, Costa Rica; (e) Dr. J. A. Martin, The Clemson Agricultural College, Clemson, S. C.; (f) Dr. E. M. Meader, University of New Hampshire, Durham, N. H.; (g) Local sources; (h) Dr. W. H. Lachman, University of Mass., Amherst, Mass.; (i) Dr. R. K. Dennett, University of Hawaii, Honolulu, Hawaii; and (j) F. Lagomarsino and Sons, Sacramento, Calif.

[b] The number of plants in each root-knot class was multiplied by the class value (healthy, 0; slight, 25; moderate, 50; severe, 75; and dead, 100), the totals added, and the sum divided by the total number of plants to obtain the root-knot index for each entry.

been placed in the most susceptible group in previous tests. Seed of California Wonder, World Beater, Oakview Wonder, Pimiento, and Ruby King varietal types obtained from various sources were planted in the test bed along with a bell breeding line (M174-B) and the resistant line Santanka xS. Replicates as such were not used since there were unequal numbers of lots within the types. Rows of a particular type were planted across the bed at regular intervals, and the resistant line, Santanka xS, was grown in every tenth row.

World Beater from 1 source had root-knot indices of 71.8, 74.3, 71.4, and 74.7 for the 4 rows planted, with an average of 129 plants classified per row. This variation from row to row was small and was consistent throughout the bed. The variation of indices for World Beater from 4 different seed sources was of the same order; 73.1, 72.2, 72.5, and 66.9, with an over-all average of 71.6. This was true of different seed sources in the other varietal types tested. Further, the differences in the types were the same (Table 4). It was apparent that all were equally susceptible.

In addition to checking on variety and seed source differences, a further purpose of the large-scale tests was to search for resistant individuals. It had been

TABLE 3.—*Angles of root-knot indices for 2 trials of 36 selected pepper varieties or strains grown in soil infested with M. incognita acrita*

Variety or strain[a]	Third trial Root-knot index[b]	Rank	Fourth trial Root-knot index[b]	Rank	Average root-knot index[b]
405B Mexico	4.1	1	10.7	1	7.4
526 "Merceh 21"	9.4	2	20.6	3	15.0
45107 Santanka xS	16.7	3	19.0	2	17.9
4575 P4	21.0	4	21.3	4	21.2
405 Mexico	27.7	6	25.6	5	26.7
P.I. 155299 Arabia	29.6	7	26.0	6	27.8
Anaheim Chile	31.0	8-9	28.4	7	29.7
Italian Pickling	31.0	8-9	28.6	8	29.8
4695 Brazil	27.2	5	34.5	11	30.9
46111 Argentina	33.9	10	29.1	9	31.5
AC 390-1	36.1	12	32.2	10	34.2
P.I. 135873 Baluchistan	35.2	11	35.8	13	35.5
Red Chili Hot	43.3	16	34.6	12	39.0
Korea 3-1947	39.9	14	39.9	16	39.9
46107 Argentina	42.5	15	37.7	14	40.1
P.I. 141927 Manchuria	39.4	13	44.3	17	41.9
P.I. 135824 Afghanistan	46.6	18	39.5	15	43.1
Red Chili	48.6	19	44.5	18	46.6
46136	51.8	20	51.1	19	51.5
4646 Bird's Eye	54.6	21	53.0	21	53.8
No. 72	46.5	17	62.4	31	54.5
4652c Louisiana Sport	59.1	27	51.7	20	55.4
Ozark Giant	57.1	22	56.9	25	57.0
Ruby King	58.6	25-26	55.6	22	57.1
4656c Hungarian Yellow Wax	58.3	24	56.0	23	57.2
California Wonder	59.7	32-33	56.2	24	57.8
46116 Mexico	58.1	23	58.6	26	58.4
Truheart	58.6	25-26	58.8	27	58.7
Oakview Wonder	59.6	30-31	59.6	28	59.6
4651 TBM Resistant	59.2	28	60.4	29	59.8
Blight Res. World Beater	60.0	34-36	60.6	30	60.3
4786 Brazil	59.6	30-31	63.2	32	61.4
4788 Peru	59.5	29	64.6	33-34	62.1
46106 Argentina	59.7	32-33	64.6	33-34	62.2
4790 Peru	60.0	34-36	65.4	35	62.7
Manatee Wonder	60.0	34-36	69.0	36	64.5
LSD 5 per cent	7.6		7.8		
LSD 1 per cent	10.1		10.3		

[a] See Tables 1 and 2 for seed sources.

[b] The number of plants in each root-knot class was multiplied by the class value (healthy, 0; slight, 25; moderate, 50; severe, 75; and dead, 100), the totals added, and the sum divided by the total number of plants to obtain the root-knot index for each entry.

noted during the trials that there were usually lesser-affected individuals in the most susceptible varieties. In many instances the roots of the apparently resistant plants were entwined with roots of plants placed in the severe class. Out of the 7,031 plants of the commercial bell types classified, 38 healthy, 53 slight, and 4 moderate were potted in infested soil taken from the test bench. They represented 72 and 28 per cent of the total plants in the healthy and slight classes, respectively.

TABLE 4.—*Root-knot indices obtained by testing large numbers of commercial-type peppers from different seed sources to M. incognita acrita*

Type or variety	No. of seed sources	No. plants tested	Average root-knot index[b]
California Wonder	7	1802	70.4
World Beater	4	1761	71.6
Oakview Wonder	1	1500	72.1
Pimiento	2	1079	71.7
Ruby King	3	726	71.4
M174B	1	163	71.8
Santanka xS[a]	1	942	25.6

[a] Used as resistant check.

[b] The number of plants in each root-knot class was multiplied by the class value (healthy, 0; slight, 25; moderate, 50; severe, 75; and dead, 100), the totals added, and the sum divided by the total number of plants to obtain the root-knot index for each entry.

After approximately 4 weeks, the plants were taken up and reclassified. In most cases (Table 5) there was an advance in the rating of the individual plants. Two plants each of Ruby King and M174B did not advance from the slight class. Only in Pimiento was there any indication that plants in the original classification were other than some form of escapes. There, 8 of 19 healthy plants were still healthy after the additional time in infested soil. The 2 Ruby King plants and the 2 M174B plants that remained in the slight class, 2 of the Oakview Wonder plants that advanced from slight to moderate, 2 of the Pimiento that advanced from healthy to slight, and 1 of the Pimiento that remained healthy were transplanted and selfed for progeny tests. When the progeny were tested in infested soil, all were susceptible except those from the healthy Pimiento. The progeny of the Pimiento gave a mixture of resistant and susceptible plants that were not true to the Pimiento type. The leaves were small and narrow and the pods were long, slender, and pungent. They evidently resulted from a mixture or an outcross.

DISCUSSION.—All of the varieties rated highly resistant were small-fruited hot peppers. Where such types are suitable for commercial use, they are worthy of trial. Insofar as bell and pimiento types are concerned, it appears that any root-knot resistance would have to be transferred to them by breeding. With the many differences in type this could be very difficult, unless genetic control of the resistance is rather simple.

A further problem that has appeared since the work was started is how the resistant lines behave to the other root-knot nematodes. This has been reported briefly (6). It appears that the resistance covers most of the root-knot forms that occur generally in the South.

Damage to commercial crops of pepper by root-knot nematodes has not been investigated. It is well known that nematodes are widely distributed, and evidence from these tests demonstrate that pepper is readily

TABLE 5.—*Root-knot ratings of pepper plants from a nematode test (M. incognita acrita) that were potted in infested soil and reclassified approximately 4 weeks later*

Type or variety	Number potted	Original class	Number rated as indicated 4 weeks later				
			Healthy	Slight	Moderate	Severe	Dead
California Wonder	13	Healthy	0	5	4	2	2
	7	Slight	0	0	3	4	0
World Beater	3	Healthy	0	0	1	2	0
	17	Slight	0	0	10	6	1
Oakview Wonder	3	Healthy	0	0	0	1	2
	10	Slight	0	0	6	3	1
	2	Moderate	0	0	2	0	0
Pimiento	19	Healthy	8	8	2	0	1
	6	Slight	0	3	0	1	2
Ruby King	9	Slight	0	2	4	2	1
	2	Moderate	0	0	0	0	2
M174B	4	Slight	0	2	0	1	1

attacked. In the field, as on many other crops, damage would probably be attritive and not total, and would contribute to an unthrifty condition. Absolute measurement of damage would require replication of fumigated and nonfumigated plots. In breeding work with the variety collection tested here, all entries have been regularly given a visual vigor rating in the field. This rating correlated well with the root-knot index after it was determined.

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INCREASING THE ABSORPTION OF STREPTOMYCIN BY LEAVES AND FLOWERS WITH GLYCEROL [1]

Reed A. Gray

SUMMARY

Glycerol was found to be an excellent agent for increasing the absorption of streptomycin by the leaves of bean, tomato, pepper and tobacco plants. A notable increase was evident in as little as 3 hours after spraying the bean leaves. In 6 hours absorption time, the addition of 1 per cent glycerol to a spray containing 500 ppm streptomycin caused a 5-fold increase in the absorption of the antibiotic by bean leaves, and in 96 hours the increase was nearly 24-fold. Glycerol also increased the absorption of streptomycin by quince flowers. Sorbitol, diethylene glycol, and other polyhydroxy alcohols were effective in increasing the absorption of streptomycin by bean leaves, but less so than glycerol. Wetting agents usually were without effect on absorption of streptomycin, except that when added to streptomycin-glycerol sprays they reduced the beneficial effect of glycerol. The absorption of 2 other antibiotics, chloromycetin and streptothricin, also was increased by the addition of glycerol, but glycerol did not seem to affect greatly the absorption of oxytetracycline and neomycin at the levels tested. Glycerol greatly increased the effectiveness of streptomycin sprays in controlling common bacterial blight of beans in greenhouse plants. This increase in effectiveness was correlated with an increase in absorption of the antibiotic by the leaves.

An agent that increases the absorption of streptomycin by plant foliage over a short period of time might increase the effectiveness of streptomycin sprays against certain plant diseases, permit use of a lower concentration of streptomycin in sprays, and eliminate the problem of the antibiotic being washed off by rain a few hours after application. Results of greenhouse tests with glycerol, which proved to be such an agent, are reported herein.

MATERIALS AND METHODS. — The absorption tests were made on Pinto bean plants growing in light soil in the greenhouse at 26°C. When first trifoliate leaves were about ⅓ fully developed, one of the twin primary leaves was sprayed on the top surface with an aqueous solution containing 500 ppm streptomycin sulfate. The spray was applied until small droplets covered the leaf but did not run off. The other twin leaf was sprayed in the same manner with a solution containing 500 ppm streptomycin sulfate and 1 per cent glycerol (U.S.P. Grade) by volume. From 4 to 6 leaves were used for each treatment. After 24 hours, the leaves were harvested and washed individually, first in a stream of water for 30 seconds and then for 15 minutes immersed in running water in a 600-ml beaker. The water was poured from the beaker and replaced with fresh water 5 times. Each leaf was then blotted dry, wrapped in aluminum foil, frozen overnight in a dry-ice chest, thawed, wrapped in cheesecloth, and pressed out in a garlic press. The juice so obtained was assayed for streptomycin content.

One-tenth ml of juice was pipetted on a filter paper disc (S&S No. 740-E), and the disc was dried at 37°C on a glass plate, then placed on an agar plate seeded with washed cells of a streptomycin-dependent strain of *Escherichia coli* (Mig.) Cast. & Chal. Samples were run in duplicate. Growth zones were measured after incubating the culture 40 hours at 28°C. Streptomycin standards were made in expressed juice of a large batch of Pinto bean leaves and run at the same time. A straight line was obtained by plotting the diameter of the growth zones against the logarithm of the concentration over a range of 1.5 to 800 ppm of streptomycin sulfate. All concentrations of streptomycin reported here are in terms of pure streptomycin sulfate.

RESULTS. — *Absorption of streptomycin by bean leaves at various time intervals and the effect of adding glycerol.*—Initial experiments showed that very little streptomycin is absorbed by Pinto bean leaves in a 24-hour period when they are sprayed on the upper surfaces with 500 or 5000 ppm of an aqueous streptomycin solution with or without the addition of a wetting agent. About 5–7 times as much streptomycin was absorbed when the lower surfaces of bean leaves were sprayed than when the upper surfaces were sprayed. The addition of 1 per cent glycerol to the spray increased streptomycin absorption, by the upper surfaces of the leaves, 10- to 30-fold in 24 hours. In an experiment on absorption at shorter time intervals, 4 leaves of each treatment were harvested 1 hour after spraying and another set 3 hours after spraying. In the 1-hour samples, no streptomycin was detected in juice of leaves sprayed with 500 ppm streptomycin alone, whereas that of leaves sprayed with 500 ppm streptomycin plus 1 per cent glycerol contained an average of 1.9 ppm streptomycin. In the 3-hour samples, 8 ppm of streptomycin was found in the juice of leaves sprayed with the glycerol-containing spray. This was twice the amount found in juice of leaves sprayed with streptomycin alone and harvested 24 hours after spraying.

In an experiment on absorption during longer periods, 48 plants were sprayed at 10:00 a.m. Six leaves

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sprayed with 500 ppm streptomycin and the 6 matched twin leaves sprayed with 500 ppm streptomycin plus 1 per cent glycerol were harvested at each of the following time intervals: 6, 12, 24, 48, 72, and 96 hours after spraying. The average concentration of streptomycin found in the 6 leaves of each treatment at each time interval was determined.

The addition of 1 per cent glycerol to the sprays containing streptomycin caused over a 5-fold increase in absorption of streptomycin in 6 hours (Fig. 1). At 96 hours after spraying, the absorption from the streptomycin-glycerol spray was 23.6 times that from the streptomycin spray alone. Absorption increased with time in the leaves sprayed with the streptomycin-glycerol combination, but there was very little increase in absorption with time when streptomycin alone was used in the spray. No injury was detected in the bean leaves at 96 hours or at 8 days after treatment.

Leaves sprayed with streptomycin solution appeared dry 1 hour after spraying. When 1 per cent glycerol was added to the spray, small shiny specks of glycerol remained on the leaves 1 hour after spraying. In 2 days, the glycerol specks had almost disappeared, and after 4 days they could no longer be seen on the leaf surfaces.

Effect of varying the concentration of glycerol and streptomycin.—The addition of 1 per cent glycerol to a spray containing 500 ppm streptomycin caused a 10-fold increase in absorption of streptomycin in a 24-hour absorption period, the addition of 0.5 per cent glycerol caused a 6.1-fold increase, and 0.25 per cent glycerol caused a 4-fold increase. These results are based on the average of 4 samples for each treatment.

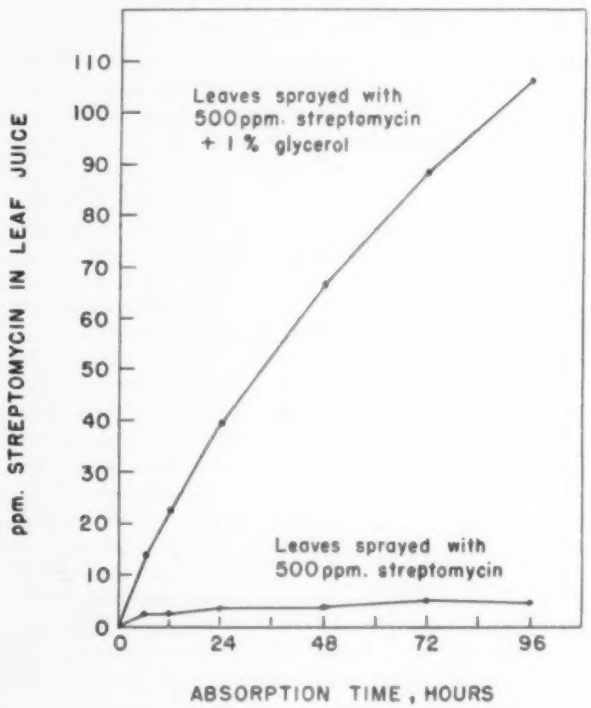

FIG. 1. Absorption of streptomycin by bean leaves sprayed with streptomycin alone and with streptomycin plus glycerol.

In another experiment, the concentration of streptomycin in the sprays was varied in sprays with and without 2 per cent glycerol. Based on the average of 3 leaves of 3 plants for each treatment, the addition of 2 per cent glycerol to sprays containing different amounts of streptomycin resulted in the following increases: 400 ppm, 16-fold; 200 ppm, 10.7-fold; 100 ppm, 2.8-fold; 50 ppm, 4.9-fold; and 25 ppm, 2.4-fold.

Effect of glycerol on the absorption of streptomycin by tomato, pepper, and tobacco leaves.—Streptomycin as a spray treatment has been reported for the control of wildfire disease of tobacco (8) caused by *Pseudomonas tabaci* (Wolf & Foster) Stevens, blue mold of tobacco (7) caused by *Perunospora tabacina* Adam, and bacterial spot disease of tomato and pepper (3) caused by *Xanthomonas vesicatoria* (Doidge) Dows. Therefore, the effect of glycerol on the absorption of streptomycin by these plants was tested. The top surface of the second and fourth leaves from the top of 2 Bonny Best tomato plants 1 ft. tall were sprayed with a solution containing 500 ppm of streptomycin, and the third and fifth leaves were sprayed with a similar solution containing 1 per cent glycerol. The leaves of 2 more plants were sprayed in the reverse order. Twenty-four hours later, the 2 leaves on each plant receiving the same treatment were harvested and combined. The leaves in each composite sample were washed and assayed for streptomycin in a manner similar to that used for bean leaves. Leaves of 4 White Burley tobacco plants were treated in the same manner. Pinto bean leaves were tested at the same time for comparison. The addition of 1 per cent glycerol to a spray containing 500 ppm streptomycin increased the absorption by tomato leaves 20 times, by tobacco leaves 31 times, and by bean leaves about 10 times (Table 1).

In a similar experiment, the addition of 1 per cent glycerol to streptomycin sprays increased absorption by California Wonder pepper leaves from a nondetectable amount (less than 1 ppm) to 7.6 ppm when a spray level of 100 ppm streptomycin was used, from a nondetectable amount to 17 ppm at a spray level of 200 ppm streptomycin, and from 1.3 to 40 ppm at

TABLE 1.—*Absorption of streptomycin by tomzto, tobacco, and bean leaves sprayed with streptomycin (500 ppm) with and without glycerol*

Kind of leaf	Streptomycin[a] in juice of leaves sprayed with streptomycin solution containing:	
	No glycerol	1 per cent glycerol
	ppm	ppm
Tobacco	1.0	30.8
Tomato	5.4	108.5
Bean	3.0	29.5

[a] Average for 4 plants. Leaves were harvested 24 hours after spraying.

a spray level of 400 ppm streptomycin. These results were obtained from the leaf samples harvested 4⅔ days after spraying. Each sample was a composite of 1 leaf from each of 8 plants receiving the same treatment. The spray containing 400 ppm streptomycin and 1 per cent glycerol caused small necrotic spots to appear on the older pepper leaves in 6 days.

The effect of glycerol on the absorption of streptomycin by quince flowers.—There is a wide potential use for the field application of streptomycin for controlling fire blight (caused by *Erwinia amylovora* (Burr.) Winslow et al) in apples (2, 5, 9, 13, 14), pears (1, 4), and quinces. Since the blossoming period is a critical one in the development of this disease, it appeared desirable to determine the effect of glycerol on the absorption of streptomycin by flowers. Quince blossoms were available from a nursery, and they were brought into a greenhouse maintained at 21°C. The flowers on 2 branches were sprayed with 25 ppm streptomycin and those on 2 other branches were sprayed with a similar solution containing 1 per cent glycerol. Both sides of the flower petals were sprayed. This procedure was repeated with higher concentrations of streptomycin (50, 100, 200, and 400 ppm). In another experiment, the blossoms were sprayed twice in the bud stage and once after they had opened. One day after the last spray, 5 whole flowers were harvested from each treatment and placed in a beaker of water, which was shaken for 20 minutes. The water was changed at 1- to 2-minute intervals. The flowers were prepared for assay and assayed for streptomycin as previously described, except that the juice was neutralized with a drop of 3 per cent ammonia water. No streptomycin could be detected in the expressed juice of quince flowers sprayed with solutions containing 100 ppm or less of streptomycin with or without added glycerol (Table 2). The addition of 1 per cent glycerol to sprays containing 200 ppm streptomycin increased the absorption 2.2 times in one experiment and from a nondetectable amount to 2.6 ppm in another experiment. The addition of 1 per cent glycerol to 400 ppm streptomycin increased the absorption by quince flowers 4.8 times, 5.4 times, and 9.2 times, respectively, in 3 different tests. Sprayed blossoms were examined daily for injury. No damage was detected in the blossoms from sprays containing as high as 400 ppm streptomycin and 1 per cent glycerol.

Effect of other compounds on the absorption of streptomycin by leaves.—Other compounds, including a number of polyhydroxy alcohols, humectants, and wetting agents were tested for their effect on the absorption of streptomycin by bean leaves. In each case streptomycin alone was compared with the streptomycin-agent mixture on the opposite twin primary leaves of the same Pinto bean plants. The tests were carried out as previously described. The leaves were harvested 24 hours after spraying. The results of several experiments carried out at different times with different groups of plants are summarized in Table 3.

The addition of wetting agents usually did not cause significant increases in absorption of streptomycin, whereas the addition of hygroscopic compounds usually increased absorption. There was some variation from one test to another. Most polyhydroxy alcohols tested increased absorption. Of the compounds tested, glycerol proved to be the most effective, followed in order by sorbitol, and diethylene glycol. Increasing the concentration of streptomycin from 500 to 5000 ppm in the sprays without additives did not increase absorption of streptomycin significantly, but it did when glycerol was present. The addition of glycerol to a spray containing 500 ppm streptomycin was much more effective than a 10-fold increase in the concentration of streptomycin in the spray.

TABLE 2.—*Absorption of streptomycin by quince flowers sprayed with streptomycin (500 ppm) with and without glycerol*

Concentration of streptomycin in sprays	Streptomycin[a] in juice of flowers sprayed with streptomycin solution containing:	
	No glycerol	1 per cent glycerol
ppm	ppm	ppm
	(1 spray on open flowers)	
25	<1	<1
50	<1	<1
100	<1	<1
200	2.3	5.6
400	5.4	26.0
	(2 sprays on buds, 1 spray on open flowers)	
25	<1	<1
50	<1	<1
100	<1	<1
200	<1	2.6
400	2.4	14.0
200	<1	<1
400	2.0	18.5

[a] Quantities less than 1 ppm were not detectable. Figures are for composite samples, each of 5 flowers. Flowers were harvested 24 hours after completion of spraying.

The mixture of 1 per cent methyl cellosolve and 1 per cent Carbowax 4000, reported by Goodman (5) to increase the effectiveness of streptomycin against fire blight in apple seedlings, did not affect absorption of streptomycin by bean leaves in this test.

Effect of glycerol on leaf absorption of antibiotics other than streptomycin.—Other antibiotics were tested on Pinto beans in a manner similar to that used for streptomycin. One per cent glycerol was added to the sprays applied to half of the twin leaves. Four plants were used for each antibiotic treatment. The leaves were harvested 24 hours later; they were washed and prepared for assay as previously described. The juice was assayed on agar plates seeded with *Bacillus subtilis* Cohn *emend.* Prazmowski, or with *Micrococcus pyogenes* var. *aureus* (Rosenbach) Zopf.

The addition of 1 per cent glycerol to a solution containing 500 ppm of the antibiotic increased absorption over 5 times in the case of streptothricin, over 9 times in the case of chloromycetin, and over

TABLE 3.—*Streptomycin content of extracts from bean leaves sprayed with streptomycin solutions with and without various additives*

Experiment	Additive used	Streptomycin[a] in juice of leaves sprayed with: Streptomycin alone	Streptomycin + additive
		ppm	ppm
	(500 ppm streptomycin in spray)		
1	1% Tween 20[b]	2.1	4.0
	2% Glycerol	1.6	41.0
	1% Tween 20 + 2% glycerol	2.8	20.0
	1% Glycerol	5.5	61.0
	0.5% Glycerol	2.5	32.0
	2% Ethylene glycol	1.0	7.8
	2% Propylene glycol	2.1	7.1
2	2% Erythritol	1.0	3.8
	2% Adonitol	2.1	7.2
	2% Mannitol	3.0	5.6
	2% D-Sorbitol	2.1	3.5
	0.5% Glycerol	2.3	7.3
	2% Diethylene glycol	4.2	14.0
	1% Sodium carbethoxymethyl cellulose	1.6	0
3	1% Methyl cellosolve + 1% Carbowax 4000[c]	1.4	1.2
	1% Glycerol	1.5	12.6
4	1% Dimethyl acetamide	1.1	1.2
	1% Dimethyl formamide	0.8	1.4
	1% Formamide	1.0	2.0
5	1% Volck Oil[d]	3.3	3.4
	(5000 ppm streptomycin in spray)		
6	1% Tween 20	3.1	3.2
	1% Span 20[e]	2.0	9.5
	2% D-Sorbitol	3.4	69
	2% Glycerol	3.2	177
7	1% Tween 20	5.6	28.2
	1% Triton X-100[f]	6.2	4.2
	1% Tween 40[g]	6.3	28.0
	2% Corn starch (cooked)	6.1	13.5
	2% Sucrose	5.7	9.3
	2% D-Sorbitol	5.0	31.7

[a] Assays were made on leaves harvested 24 hours after spraying. Each figure is the average for extracts from 4 plants.

[b] Tween 20 (polyoxyethylene sorbitan monolaurate) is a surface active agent manufactured by Atlas Powder Co.

[c] Carbowax 4000 (polyethylene glycol) is manufactured by Carbide and Carbon Chemicals Co.

[d] Volck Oil is a highly refined oil spray manufactured by California Spray Chemical Corp.

[e] Span 20 (sorbitan monolaurate) is a surface active agent manufactured by Atlas Powder Co.

[f] Triton X-100 (alkyl aryl polyether alcohol) is a surface active agent manufactured by Rohm & Haas Co.

[g] Tween 40 (polyoxyethylene sorbitan monopalmitate) is a surface active agent manufactured by Atlas Powder Co.

16 times in the case of streptomycin (Table 4). Oxytetracycline and neomycin were not detected in the leaf juice at the levels tested, with or without added glycerol.

Activity of streptomycin-glycerol mixtures against common bean blight.—Mitchell et al (11, 12) and Zaumeyer et al (15) have reported on the effectiveness of streptomycin in controlling halo blight of beans, caused by *Pseudomonas phaseolicola* (Burk.) Dows., and common blight of beans, caused by *Xanthomonas phaseoli* (E. F. Sm.) Dows. Streptomycin was less effective against common blight than against halo blight. Marlatt (10) reported that 4 applications of aqueous solutions containing 240 and 1000 ppm of streptomycin failed to control common blight in Pinto beans in the field.

TABLE 4.—*Absorption of antibiotics other than streptomycin by bean leaves sprayed with the antibiotics with and without glycerol*

Antibiotic[a]	Antibiotic[b] in juice of leaves sprayed with: Antibiotic alone	Antibiotic + 1% glycerol
	ppm	ppm
Streptothricin	<5	27
Chloromycetin[c]	<6	55
Streptomycin	<3	49
Oxytetracycline[d]	<6	<6
Penicillin	5.3	6.3
Neomycin	<1.5	<1.5

[a] Each antibiotic was used at a concentration of 500 ppm.

[b] Each figure is the average of 4 replicates. A figure preceded by < indicates that no antibiotic was detected and the figure is the lowest concentration of the antibiotic that gave an inhibition zone in the standards.

[c] Chloromycetin is D(−)-*threo*-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide.

[d] Oxytetracycline is 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

Tests were conducted on the effect of the addition of glycerol to streptomycin sprays on the effectiveness of this antibiotic in controlling common bean blight. A preliminary account of this work has been published (6). Fifty-six Pinto bean plants with first trifoliate leaves about half expanded were used. The top surface of 24 trifoliate leaves of 8 plants were sprayed with 50 ppm streptomycin sulfate solution until tiny droplets formed. The primary leaves were

TABLE 5.—*Comparison of streptomycin and streptomycin-glycerol mixtures for the control of common bean blight*

Spray treatments[a]	Average no. of lesions on 24 leaflets 7 days after inoculation	Streptomycin[b] in juice of primary leaves
		ppm
Control, untreated	191	<1.5
50 ppm streptomycin	143	<1.5
50 ppm streptomycin + 1% glycerol	37	1.9
100 ppm streptomycin	100	<1.5
100 ppm streptomycin + 1% glycerol	18	4.9
200 ppm streptomycin	107	<1.5
200 ppm streptomycin + 1% glycerol	8	10.1

[a] One spray was applied 3 days before inoculation and the leaves were washed off just before inoculation.

[b] <1.5 indicates that no growth zone was obtained; 1.5 ppm was the lowest concentration giving a detectable zone in the standards.

sprayed for absorption studies. A second row of 8 plants was similarly sprayed with a solution containing 50 ppm streptomycin sulfate and 1 per cent glycerol. This procedure was repeated with higher concentrations of streptomycin (100 and 200 ppm), with and without glycerol. One row of 8 plants remained untreated as controls. Three days after spraying, the antibiotic was washed off the surface of the leaves with several sprays of water, and the leaves were blotted dry with a cotton cloth. The first trifoliate leaflets were then sprinkled with Carborundum and rubbed with a suspension of 2-day-old cells of *X. phaseoli* grown in yeast-extract dextrose medium in shake flasks. Lesions appearing on the under surface of the leaves were counted 7 days after inoculation. The primary leaves of the same plants were harvested at the same time (10 days after spraying). A composite sample, consisting of 1 leaf from each of the 8 plants of each treatment, was assayed.

Streptomycin alone in concentrations as high as 200 ppm caused very little reduction in symptom development, whereas a solution containing 50 ppm streptomycin and 1 per cent glycerol caused a marked reduction in the number of lesions produced by the common bean blight organism (Table 5). One spray of a solution containing 200 ppm streptomycin and 1 per cent glycerol gave almost complete control of the disease. This high degree of control was still evident 2 weeks after the spray was applied. The increase in effectiveness against the disease caused by glycerol was correlated with an increase in absorption of streptomycin by the leaves.

In another experiment, the leaves were sprayed in the same manner at 11 a.m. and again at 5 p.m. the

FIG. 2. Symptoms of common bacterial blight on Pinto bean leaves 7 days after inoculation. A) Sprayed with a solution containing 200 ppm streptomycin. B) Sprayed with a solution containing 200 ppm streptomycin and 1 per cent glycerol. C) Untreated. D) Sprayed with a solution containing 200 ppm streptomycin and 0.5 per cent glycerol.

same day. The next day the leaves were washed for 10 minutes under a water sprinkler, blotted dry, and inoculated with *X. phaseoli* as described in the last experiment. Twelve trifoliate leaves on 4 plants were used for each treatment. The total numbers of lesions appearing on the under surfaces of the leaves 6 days after inoculation for the different spray treatments were as follows: control, 3845; 200 ppm streptomycin, 2160; 200 ppm streptomycin plus 0.5 per cent glycerol, 210; and 200 ppm streptomycin plus 1 per cent glycerol, 71. Photographs of the leaves from the different treatments in this experiment are shown in Figures 2 and 3. The addition of as little as 0.5 per cent glycerol to a spray containing 200 ppm streptomycin caused a further reduction of 90 per cent in the number of lesions.

DISCUSSION.—In some of the early experiments, it was found that the lower surfaces of bean leaves absorbed about 5 to 7 times as much streptomycin as the upper surfaces. This difference may be attributed to the stomatal openings appearing only on the lower surfaces of bean leaves, or to the differences in the thickness of the cuticle on the 2 surfaces. The upper and lower surfaces of tomato leaves absorbed about the same amount of streptomycin.

The mechanism by which glycerol increases the absorption of streptomycin is thought to be the keeping of streptomycin in solution and in close contact with

FIG. 3. Symptoms of common bacterial blight on Pinto bean leaves 11 days after inoculation. A, B) Sprayed with 200 ppm streptomycin. C, D) Sprayed with 200 ppm streptomycin plus 1 per cent glycerol.

the leaf surface for a long period of time. As the water evaporates from the tiny droplets, a concentrated solution of the streptomycin in glycerol and water remains on the leaf. Very little streptomycin is absorbed before most of the water evaporates. The low volatility of the glycerol permits the glycerol to remain on the leaves for several days. Perhaps some of the glycerol is absorbed by the leaves along with the streptomycin, although no evidence has been obtained that this is so. Also, it appears possible that glycerol may increase the permeability of the leaf epidermis to streptomycin. Sorbitol and other polyhydroxy alcohols with low volatility and hygroscopic properties also were effective, but they were less so than glycerol.

The effect of glycerol on absorption is not specific for streptomycin, since the absorption of some other antibiotics (chloromycetin and streptothricin) also was increased by the addition of glycerol. It is possible that glycerol might prove useful for increasing the absorption of certain other agricultural chemical sprays, such as urea and other fertilizers, minor elements, systemic insecticides, fungicides, and plant-growth regulators.

There seemed to be considerable variability from one experiment to another in the amount of streptomycin absorbed by leaves sprayed with streptomycin-glycerol mixtures. The increase varied from 10- to 30-fold in different experiments at the same spray concentration. This variation was thought to be due to differences in light intensity and in age and condition of the plants. Less absorption occurred in young leaves that were wetted readily by the droplets; the droplets spread out and ran off the leaves easily instead of clinging to the leaves as was the case in slightly older leaves. Also, the addition of a wetting agent to streptomycin-glycerol sprays reduced the effectiveness of the glycerol.

Although a 1 per cent glycerol spray caused no phytotoxicity to bean plants, it did cause a few dead spots to appear on older tobacco leaves. Small tobacco seedlings and young leaves of older plants were not injured. It may be that glycerol causes this effect only when it enters a puncture or injured spot on the leaf, since only 1–2 injured spots occurred on a leaf sprayed with approximately 100 droplets.

The results show that increasing the absorption of streptomycin by bean leaves with glycerol is correlated with an increase in effectiveness of the antibiotic against the common bean blight disease. It seems reasonable to assume that the addition of glycerol should also increase the effectiveness of streptomycin against other plant pathogens that infect stems, leaves, or flowers.

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CONTROL OF THE HELMINTHOSPORIUM BLIGHT DISEASES ON SWEET CORN IN SOUTH FLORIDA [1]

R. S. Cox [2]

SUMMARY

Compared with the standards (nabam + zinc sulfate and zineb), several other fungicides showed promise in the control of the Helminthosporium leaf blight diseases. Formulations of maneb were particularly outstanding. Ziram, Vancide Z-65, and C&C fungicide #7443 were also effective. A high negative correlation between disease severity and yield was obtained in tests where southern corn leaf blight and northern corn leaf blight were separately involved. Thus, a uniform system for scoring for these diseases is suggested. It is postulated that a simplified disease control program might be possible with varieties normally producing 1 instead of 2 ears to the stalk. In addition to increased yields, another benefit derived from an effective control program is that of the production of a fancier grade of corn.

The Helminthosporium leaf blight diseases provide one of the most serious limiting factors in the economical production of high quality sweet corn in south Florida. Growers usually invest $20 to $30 per acre in fungicides in an attempt to control these diseases. Although an effective control program has been developed (6), it nevertheless becomes necessary for experiment station personnel to search continuously for more efficient fungicides, and to develop refinements in methods and timeliness of applications in order to improve on disease control and to reduce cost of the program.

The 2 diseases, northern corn leaf blight (caused by *Helminthosporium turcicum* Pass.) and southern corn leaf blight (caused by *H. maydis* Nisikado & Miyake) are seasonal in occurrence in south Florida. Southern leaf blight is dominant in the fall crop and may appear again in late spring. Northern leaf blight is dominant during the most important part of the growing season, i.e. late winter and spring. This seasonal occurrence, believed to be controlled primarily by temperature (6), provides an excellent opportunity for comparative studies on the effects of fungicides on these 2 diseases. This is a report on such work during the 1954-55 season.

MATERIALS AND METHODS.—Golden Security was the variety used in both experiments. Fungicides were applied with spray equipment described elsewhere (3). One overhead and 2–6 drop-nozzles, depending on the height of the corn, were used. Fifteen-in. intervals (vertically) were employed between all nozzles. Gallonage output, approximated on an acre basis, ranged from 65 to 190. The output at any given operation was controlled by the number of nozzles used. All other variables, i.e. ground speed, pressure (300 p.s.i.), and orifice size (number 3 disc in Meyer's Jumbo nozzle) remained constant. Insecticides were applied in a separate operation.

Spray applications were made twice weekly. For the fall test (plots seeded August 18, 1954), 15 sprays were applied during the period August 26–October 14. The spring plots (seeded March 7) received 12 sprays from March 30 through May 10.

In both tests, single-row plots were used in randomized blocks replicated 4 times. Plots were 40 ft. long in the fall test and 50 in the spring test. No guard rows were used in the fall test, but the rows were spaced 6 ft. apart. In the spring test, each plot row was protected by an unsprayed guard row on either side; row spacing was 3 feet. The latter arrangement was considered more satisfactory for 2 reasons: 1) the guard rows served as "traps" for spray drift, and 2) better pollination was obtained in the more closely spaced rows.

A poor stand obtained in the fall planting, primarily because of wireworm damage, resulted in low variable yields. (Note the high (30–44 per cent) coefficients of variation in Table 1.) This variability was accentuated by the adverse effects of copper spray drift from plot to plot. In the spring test, however, a very uniform stand (50 plants/plot) was obtained, and no phytotoxic materials were used. This resulted in considerably higher and more nearly uniform yields (Table 2).

The fall corn was graded by commercial standards. For the spring crop, however, observations indicated that practically all of the marketable corn would fall in the top (fancy) grade; therefore, commercial grades were not used. Rather, yield records were based on position of the ear on the stalk (Golden Security normally produces 2 to the stalk under favorable growing conditions).

An attempt was made to use a uniform system for rating both diseases. A score card based on that developed by Horsfall and Barratt (5) was used, and plots were scored for disease severity (percentage of leaf area destroyed) rather than disease incidence (number of lesions per plant). Severity ratings were

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[2] The writer is indebted to E. H. King for preparation of the graphs and to Dr. R. A. Conover for helpful suggestions in the preparation of the manuscript.

TABLE 1.—*Development of southern corn leaf blight and ear yields in sweet corn plots sprayed with various fungicides*

Material[a]	Concentration in 100 gal.	Disease severity[b]	Yield (lb.)[c]		
			Total	Fancy	#1's
None (check)		7.7	4.7	2.1	2.6
Dithane D-14 + 36 per cent zinc sulfate	2 quarts + ¾ lb.	2.0	9.4**	7.3*	2.1
LO-738	2 lb.	2.7	6.7	3.7	3.0
Fermate	2 lb.	3.0	7.7	4.4	3.3
Vancide Z-65	2 lb.	3.0	7.1	4.3	2.9
Dithane M-22	1½ lb.	3.2	8.4*	5.2*	3.2
Zerlate	2 lb.	3.2	8.3*	5.5*	2.7
C & C Exp. #7443	2 lb.	3.7	8.2*	4.3	3.9
Dithane Z-78	2 lb.	3.7	7.6	4.6	3.0
Vancide MX 427	2 lb.	4.0	8.2*	3.7	4.5
XSF.3	1 quart	5.0	6.0	3.2	2.8
Orthocide 50-W	3 lb.	6.0	7.6	4.1	3.5
Mathieson 275	2 lb.	8.0	4.6	3.0	1.6
Phygon XLN	½ lb.	8.2	3.3	2.1	1.2
LSD 0.05			3.1	2.7	N.S.
LSD 0.01			4.1	N.S.	N.S.
Coefficient of variation			30%	44%	44%

* Significant increase over the check at the 5 per cent level.
** Significant increase over the check at the 1 per cent level.
[a] Active ingredients and percentage composition of materials listed are as follows: Dithane D-14, 19 per cent nabam or disodium ethylenebis[dithiocarbamate]; LO-738, 65 per cent ethylenebis thiuramtrisulfide; Fermate, 76 per cent ferbam or ferric dimethyldithiocarbamate; Vancide Z-65 (65 per cent zinc dimethyldithiocarbamate and a zinc derivative of 2-mercaptobenzothiazole); Dithane M-22, 70 per cent maneb or manganous ethylenebis[dithiocarbamate]; Zerlate, 65 per cent ziram or zinc dimethyldithiocarbamate; C & C exp. #7443, 70 per cent active ingredient of "a complex sulfur-containing compound of undisclosed composition"; Dithane Z-78, 65 per cent zineb or zinc ethylenebis[dithiocarbamate]; Vancide MX 427, 75 per cent manganese dimethyldithiocarbamate and 2-mercaptobenzothiazole; XSF.3, silver salicylate; Orthocide 50-W, 50 per cent captan or *N*-(trichloromethylthio)-4-cyclohexene-1,2,-dicarboximide; Mathieson 275, 75 per cent pentachloronitrobenzene; Phygon XLN, 50 per cent dichlone or 2,3-dichloro-1,4-naphthoquinone. Three materials are not listed because they caused severe injury and reduced the yield to zero. These were basic copper sulfate (53 per cent metallic copper), Cop-O-Zink (basic copper sulfate and zinc oxysulfate; 42 per cent metallic copper + 11 per cent zinc), Cop-O-Zim (basic copper chloride, basic copper sulfate, zinc oxysulfate; 33 per cent metallic copper + 8 per cent manganese and 3 per cent zinc).
[b] Scored October 19, 1954, on a 0–11 scale where 0 indicates no disease and 11, complete defoliation.
[c] Harvested on October 23, 1954, and graded according to U. S. standards.

preferred over incidence ratings, since it was believed that crop yield was correlated with healthy leaf area. It would be inperative to differentiate between severity and incidence when comparing *H. turcicum* with *H. maydis*, since lesions produced by the latter are much smaller.

EXPERIMENTAL RESULTS.—*Fall test.*—Southern corn leaf blight (caused by *H. maydis*) developed in epiphytotic proportions almost immediately following seedling emergence and so continued throughout the season. It was evident that yield was correlated with disease severity (Table 1, Fig. 1). One interesting result was the apparent superior performance of nabam plus zinc sulfate over other materials, including zineb, from the standpoints of both disease control and yield, particularly of the fancy grade; another, the excellent disease control and high yields from plots treated with maneb and ziram; and a third, the failure of maneb to cause noticeable injury (Conover (2) has reported serious injury with maneb on corn in the Homestead area).

Two neutral coppers (basic copper sulfate and basic copper sulfate plus basic copper chloride) used in this test caused serious injury, reducing the yield to zero.

Spring test.—Since the first 4 months of 1955 were dry in South Florida[3], moderate disease development resulted. Initial disease symptoms, for example, were not observed until March 30, the 23rd day following seeding. After that, northern corn leaf blight (caused by *H. turcicum*) gradually increased in the untreated plots so that disease severity was rated at 6 (0–11 basis) by April 27. Normally the disease would progress rapidly in severity with such an inoculum potential; whereas the indices in the check plots on May 5, 12, and 19 actually were 6, 4.5, and 5, respectively. Thus, instead of a "build-up", the plots appeared to "grow out" of the disease during this period. This undoubtedly was due to prevailing dry weather[4].

Preliminary observations indicated that the first (upper) ears on the stalk were not adversely affected by the disease, even in the untreated plots. On the other hand, the disease did appear to affect development of the second (lower) ears. Yield records later confirmed this observation (Table 2).

A correlation between disease control and yield was

[3] Total rainfall for this period in 1955 was 6.7 in., as compared to the 30-year average of 9.6 in.; more than 20 in. have been recorded for this period.

[4] Rust (caused by *Puccinia sorghi* Schw.) appeared in the plots but failed to progress after April 20. In general, the materials that controlled blight also controlled rust.

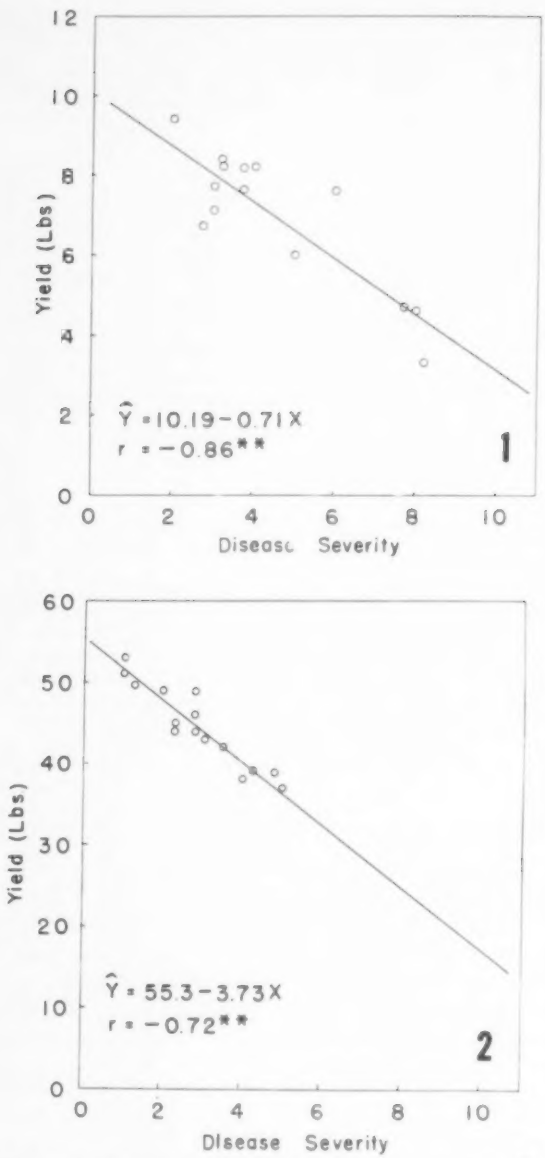

Fig. 1–2. Repression of yield on disease severity.—Fig. 1. Southern corn leaf blight.—Fig. 2. Northern corn leaf blight.

again obtained (Table 2, Fig. 2). Actually, the visually-scored disease indices are believed to reflect fungicide efficacy more accurately than do the yield data; the latter are necessarily influenced by factors other than disease.

The maneb-sprayed plots consistently showed better disease control than did the other plots throughout the season. This difference was particularly noticeable during the week following April 27—the period when the disease was most active. As in the fall test, this material caused no noticeable injury.

Nabam plus zinc sulfate appeared to be superior once again to zineb, although both effected highly significant disease control, which in turn was reflected in highly significant yield increases over that of the check. Two ziram formulations also gave good control, as did ferbam, Vancide Z-65, Carbide and Carbon 7443, and Vancide MX 427 (Table 2).

There are many hidden values in a disease control program, such as improved quality and grade of horticultural and agronomic crops, that are frequently difficult to measure. An opportunity was afforded during this work to gain information on some of these. Table 2 shows that use of effective fungicides resulted in significant increases in weight of the second ear. This increase apparently was due largely to a better fill toward the tip of the ear. The significance of this probably does not lie entirely in the increased yield (weight) per acre. The more eye-appealing display that the fuller ears would provide on the market counter should be taken into consideration in analyzing the value of an effective control program.

Discussion.—There are several factors that might hinder the development of comparable score cards for the 2 diseases in question. First, the considerable difference in lesion size of the 2 conceivably could affect visual acuity. One might be prone to overrate the smaller-lesioned disease (southern leaf blight), i.e. score more for incidence rather than severity. Secondly, Eldredge (4) has shown that the effect of mechanical leaf damage on yield is closely tied in with the stage of maturity of the corn at which the damage is sustained. Thirdly, Chester (1) has objected to use of the Horsfall and Barratt (5) system as a means of correlating disease with yield on the grounds that sufficient weight is not given to the middle (20–80 per cent) portion of the disease spectrum. Finally, varietal response might be a fourth factor to consider.

In spite of these potential difficulties, a comparison of the regression lines in Figures 1 and 2 shows that the disease indices for the 2 tests were comparable. Theoretically, plots scored with a disease severity rating of 5 in both cases would have yielded approximately 65 per cent of that expected in the absence of disease.

Another comparison is the high negative correlation between disease severity and yield (86 per cent for southern leaf blight and 72 per cent for northern leaf blight). Thus it would appear that if Chester's objection is valid, it could be obviated by scoring for disease severity rather than incidence in the case of the corn leaf blights. (The picture probably would be more complex where damage other than necrosis, i.e. systemic action, dwarfing, dieback, etc., is involved.) If further work confirms the present findings, it is suggested that comparable score cards for the 2 diseases could be developed for estimating yield losses.

The effect of the disease on ear formation under the moderate disease conditions of the spring test was also noteworthy. Apparently there was sufficient healthy leaf area to support normal formation of the first ear, even where fungicides were not used (Table 2). This obviously was not the case with the second ear. Thus, with a one-eared variety of equal disease

TABLE 2.—*Development of northern corn leaf blight and ear yields in sweet corn plots sprayed with various fungicides*

Material[a]	Concentration in 100 gal.	Disease severity[b] 5/19/55	Yield (lb.)[c] Total	First (upper) ear	Second (lower) ear	Ear size (lb.) First (upper)	Second (lower)
None (check)		5.0	37	28	9	0.59	0.52
Dithane M-22	1½ lb.	1.1	53**	33	20**	0.61	0.55*
Manzate	1½ lb.	1.0	51**	32	19**	0.60	0.55*
Dithane D-14 + 36 per cent zinc sulfate	2 quarts + ¾ lb.	1.3	50**	29	21**	0.63	0.55*
Dithane Z-78	2 lb.	2.0	49**	32	17*	0.61	0.55*
Zerlate	2 lb.	2.8	49**	30	19**	0.63	0.56*
Vancide Z-65	2 lb.	2.8	46**	29	17*	0.61	0.51
C & C 7443	2 lb.	2.3	46**	29	17*	0.58	0.54
Fermate	2 lb.	2.8	44*	29	15*	0.60	0.53
Z-C Spray	2 lb.	2.3	44*	28	16*	0.61	0.54
Vancide MX 427	2 lb.	3.1	43*	27	16*	0.60	0.56*
Orthocide 50-W	3 lb.	3.5	42	27	15*	0.62	0.54
Agri-mycin 100	200 ppm	4.3	39	28	11	0.61	0.51
XSF.3	3 pints	4.8	39	30	9	0.60	0.50
Vancide F-1655W	2 lb.	4.0	38	26	12	0.59	0.52
LSD 0.05			6	N.S.	6	N.S.	0.03
LSD 0.01			8	N.S.	9	N.S.	N.S.
Coefficient of Variation			8%		16%	5%	3%

* Significant increase over the check at the 5 per cent level.
** Significant increase over the check at the 1 per cent level.
[a] Active ingredients and percentage composition of materials not listed under footnote [a] Table 1: Manzate, 70 per cent manganous ethylenebis[dithiocarbamate]; Z-C spray, 76 per cent zinc dimethyldithiocarbamate; Agri-mycin 100, 15 per cent streptomycin sulfate and 1.5 per cent oxytetracycline (4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide; Vancide F-1655W, 75 per cent ferric nitroso dimethyldithiocarbamate.
[b] Scored May 19, 1955, on a 0–11 basis where 0 indicates no disease and 11. complete defoliation.
[c] Harvested May 24, 1955.

susceptibility, it is postulated that considerably fewer —perhaps no—fungicide applications would be necessary under conditions of mild to moderate disease. Another desirable feature of a single-eared stalk would be that of a more uniform maturity date; the first ears normally mature earlier than the second ones. With the present trend toward mechanization in south Florida, a single harvest operation is mandatory. This must necessarily impair over-all quality where 2 ears per stalk are involved.

Whether this possible 2-fold advantage, i.e. a less strenuous disease control program and improved quality in the ear of a single-eared stalk, outweighs such expected disadvantages as reduced yields remains an important point to be determined.

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KERNEL SMUT OF BIG BLUESTEM [1]

John Dunleavy [2]

SUMMARY

The most pronounced symptom exhibited by *Andropogon gerardi* when infected with the fungus *Sphacelotheca occidentalis* is severe stunting of the plant. Silver-gray linear sori ½–1 cm in length develop from the pistils of infected plants and become dark brown at maturity. The perennial fungus overwinters in the rhizomes of infected grass plants that deteriorate and die in a few years. A low percentage of infection was obtained when the rhizomes of plants were inoculated with suspensions of chlamydospores and sporidia.

Histological studies revealed that the fungus hyphae were distributed throughout the rhizomes, stems, and flowers of mature plants. The fungus was found in parenchyma cells only; it was never identified in meristematic tissue. As chlamydospores developed in the pistils, the pistil tissue elongated rapidly and was replaced externally by a hyphal mat of fungus cells. As sorus development progressed, a layer of chlamydospores was produced beneath the hyphal mat. A central column of pistil tissue remained intact in the fully developed sorus.

Good fungus growth was obtained on sterilized soil rich in manure and on other common media. Optimum temperature for growth of monosporidial lines varied from 25° to 33°C. Optimum pH for growth was 6.0. Optimum temperature for chlamydospore germination was between 26° and 28°C.

Chlamydospores from 3 different locations germinated best on 3 different media. Monosporidial isolates from the 3 spore collections differed in cultural characters.

Kernel smut of big bluestem is a disease caused by *Sphacelotheca occidentalis* (Seym.) Clint. and occurs only in Canada and the United States (2, 9). Besides *Andropogon gerardi* Vitm. (big bluestem), the fungus has been reported to infect *A. glomeratus*, *A. halli*, *A. scoparius*, *A. virginicus*, and *Heteropogon contortus*. This study deals with *A. gerardi* only.

The most pronounced field symptom exhibited by *A. gerardi* when infected with *S. occidentalis* is severe stunting of the plant. Healthy panicles normally reach a height of 4–6 ft., whereas smutted panicles range from less than 1 in. to 3 ft. in height (Fig. 1). The linear sori that develop from the ovaries are at first silver-gray and become dark brown at maturity. They are ½ to 1 cm in length and are covered by an evident membrane that dehisces from the apex, disclosing a powdery mass of spores surrounding a central columella. The sterile cells composing the membrane are hyaline, are variable in size and shape, and are often obscured through semi-gelatinization. The reddish-brown chlamydospores are ovoid to spherical, and often adhere to one another, but they do not form spore balls (5).

Since no record of the life cycle of this fungus was found in the literature, this study was undertaken to learn more of the etiology of the disease. The investigations centered around field and greenhouse studies to determine the mode of infection and overwintering. A histological study was made to determine the plant parts infected and the mode of hyphal penetration through these tissues.

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MATERIALS AND METHODS.—Plants used in this study were taken as sods from local pastures or were grown from a commercial grade of seed harvested in north central Nebraska. All seed was disinfected by soaking in a 1:10 dilution of sodium hypochlorite for 15 minutes, unless otherwise stated.

Dormancy was induced in sods by allowing them to dry for 3–4 weeks. The tops were then cut off 2–3 in. above the soil. Dormancy was broken by watering the plants very lightly and placing them in a refrigerator at 5°C for 2 weeks, after which the temperature was lowered to 1°C for 2 additional weeks. The plants were then placed in the greenhouse and watered regularly.

Inoculum consisted of chlamydospores and sporidia. When suspensions of chlamydospores were used, the spores from 1 sorus approximately 1 cm long were added to 1 liter of distilled water.

Floral inoculations were made by applying the inoculum to the flowers by means of a small brush. Growing points were inoculated by means of a hypodermic syringe. Some seed and seedlings were inoculated by the use of a vacuum pump to obtain a vacuum pressure of 25 lb. Material being inoculated was placed in a wide-mouth bottle and covered with a suspension of spores. Vacuum was applied 3 times for 3-minute periods for each inoculation.

RESULTS.—*Overwintering of the fungus.*—The recurrence of smutted plants in the same areas in succeeding years indicated the presence of a perennial mycelium. Twenty-six sods known to be infected, together with 10 healthy sods, were removed from a pasture shortly after the plants had flowered. Dormancy was induced and broken, and all plants survived the treatment. The first smutted plant flowered 8 weeks after removal from the refrigerator, and all but 2 infected sods produced smutted flowers. The 2 sods that did not produce smutted flowers were composed

of severely stunted plants that did not produce any flowers. All 10 check plants produced normal flowers. After the plants had flowered, the sods were dried for several weeks and dormancy was again broken. After this treatment, 1 infected sod failed to produce any plants. The 2 infected sods whose plants failed to flower after the first treatment failed to flower again. In addition, an infected sod that had produced flowers after the first treatment failed to produce them after the second treatment. All 10 check plants flowered.

The same procedure was used to induce dormancy a third time. Six of the 25 infected sods produced no plants. Eight infected sods, in addition to the 3 previously mentioned, failed to produce plants that flowered. All healthy plants flowered.

Dormancy was induced in 6 plants that had been artificially inoculated. During the last 2 weeks of the treatment, during which dormancy was broken, the temperature was lowered to −20°C instead of 1°. The lower temperature had no ill effect on either the mycelium or the plants. All plants survived the treatment, and all produced smutted heads.

Characteristically, the first smutted shoots to appear after dormancy had been broken arose from the first node of plants smutted previously. Subsequently smutted shoots arose directly from the rhizomes.

Results indicate that dormancy may be broken in *A. gerardi* by 1 month of refrigeration and that *S. occidentalis* is a perennial smut that overwinters in infected rhizomes of the host plant and can withstand temperatures as low as −20°C. After 3 dormant periods, the fungus had killed 44 per cent of the infected sods and had inhibited 38 per cent of the infected sods from producing plants bearing flowers.

Method of infection.—Field observations revealed that healthy plants are often associated with smutted plants though the smutted plants are found in isolated areas within a field. Four sods containing healthy and smutted plants were potted in 2-gal. crocks and placed in the greenhouse after dormancy had been induced and broken. Chlamydospores from the smutted flowers were allowed to contact the healthy flowers, after which the sori were placed on the surface of the soil in the crock. This entire procedure was repeated 3 times on the same plants without any of the healthy plants becoming infected.

When these plants were removed from the field, the roots and rhizomes of infected plants were very poorly developed in comparison with those of the healthy plants. Further observation of infected plants in the greenhouse substantiated the original observation.

A series of hypodermic injections of both field and greenhouse plants was made. Suspensions of sporidia or chlamydospores were introduced into the vascular system of the stem until inoculum was forced out through the apex of the tiller. Some of the tillers

FIG. 1. Typical stunting of smutted big bluestem plant. Normal plants commonly attain a height of 6 ft. The sori on the heads to the left are mature, whereas those on the heads to the right are just maturing.

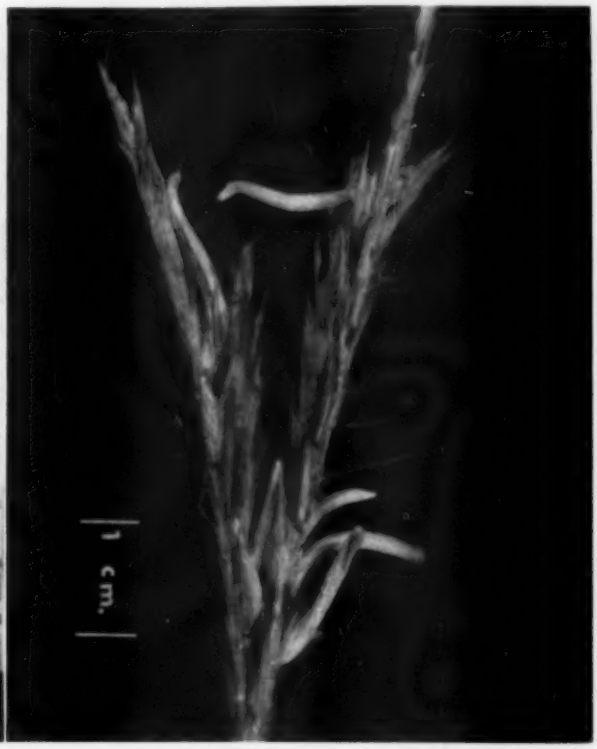

FIG. 2. A smutted head of big bluestem with exceptionally large sori. The sorus at the upper left is about to rupture.

to be inoculated were wounded by passing a needle through the stem in 6 to 8 places near the growing point. All inoculated tillers flowered, but none became smutted. The yield of seed was very poor, but it was saved for later infection studies. Dormancy was induced and broken in all greenhouse plants, and neither the tillers that developed after dormancy nor those that developed in the field the following spring were smutted.

A number of methods were used in attempts to infect the rhizomes and young shoots of big bluestem plants. Only the following method proved successful, and this method gave a low percentage of infection. Twenty plants, 5 months old, were removed from their pots and their roots washed and dipped in a suspension of sporidia. The plants were then potted in sterilized soil rich in manure. Ten ml of sporidial suspension was poured on the soil around the rhizomes before they were covered. Seven check plants were treated similarly, but were not inoculated. Five months after inoculation, 3 plants showed smutted heads. One infected plant was exceptional in that the sori were extremely large (Fig. 2). The spores within these sori were, however, of normal appearance and size. None of the check plants were infected. When the experiment was repeated, with 3-month-old plants, none of the 18 inoculated plants nor of the 9 control plants were smutted. The third time the experiment was repeated, 2 of 20 plants 5 months old were smutted and again the control plants remained noninfected. The fourth time this procedure was repeated, a suspension of chlamydospores collected from the sori on plants inoculated in the first attempt to infect the rhizomes was substituted for the suspension of sporidia. Thirty plants, 3 months old, were inoculated. Only 1 of the inoculated plants was smutted.

Numerous attempts to obtain infection of flowers, seed, and seedlings failed. Inoculated flowers were hand pollinated to assure good seed-set, but seedlings from seed that developed from inoculated flowers were all healthy. Seedlings were not infected when seed was dusted with chlamydospores and germinated. Seed inoculated with chlamydospores by the method of Kolk (6) or with a water suspension of chlamydospores in a partial vacuum likewise failed to produce diseased plants.

Histological studies.—A histological study was made to determine which host tissues are invaded by the fungus. Frozen pieces of various parts of infected plants were sectioned by means of a sliding microtome. Young tissues were sectioned 5 μ thick, whereas older tissues were sectioned 10 μ thick. Infected plants in different stages of development were studied in an attempt to discover how the fungus reached and infected the flowers of the host.

A number of the more widely used stains were tested in an attempt to find one that will differentiate clearly fungus from host tissue. Cotton blue proved to be the best stain for this purpose. Host tissues were not stained except when the sections were to be photographed, in which case safranin "O" was used.

Sections were made at intervals along the plant axis from the rhizomes to the infected flowers. Hyphae at the base of buds occurring along the rhizome indicated that the initial infection of the shoot occurred in the meristematic region of the rhizome before the differentiation of the shoot. Fungus hyphae were found in the rhizome, stem, and rachis of all infected shoots regardless of age. The hyphae were less frequently observed, however, in the rachis than in the other parts of the plant.

The hyphae were intracellular and were found in parenchyma ground tissue located between the vascular bundles. They usually had a corkscrew appearance. Subepidermal parenchyma cells also were frequently observed to be invaded by the fungus. These cells were considerably smaller than the more centrally located parenchyma cells. A few of them appeared to be almost completely packed with hyphae. In 1 instance, hyphae were located in cells near the leaf base and in the lamina.

Characteristic hyphae never were observed in either the growing points of shoots or in the undifferentiated meristematic tissues of pistils. Careful study of the slides indicated that very small spherical hyphal cells, 1–5 μ in diameter, might be present in these tissues. This could not be substantiated, however, because of the fact that certain cell inclusions also took the cotton blue stain, thus making positive identification impossible.

In order to study the infection of the pistil, immature infected flowers were removed from the boot, and the pistils were dissected out in the field of a dissecting microscope. The first indication of infection of a pistil was a slight browning, in the shape of a cone, at the apex. This discoloration was within the tissues. As increasingly older pistils were examined, it was found that this discolored area expanded with the development of the pistil until the pistil had lost all semblance of a normal plant organ.

These pistils were then imbedded in paraffin and cut in 5- to 10-μ sections and stained. Observation of sections of young pistils indicated that the discoloration within the pistils was due to the formation of chlamydospores. Formation of chlamydospores was accompanied by the appearance of compact masses of hyphae that cut off elongate cells. These cells had enlarged considerably and had changed to a light brown color as the walls thickened. Very small spherical cells similar to those found in meristem cells of the pistil before chlamydospore formation were observed in and near hyphal masses that had been actively engaged in chlamydospore formation.

Sections of older pistils showed that as the young sorus within the pistil elongated, a hyphal mat gradually replaced the outer covering of pistil tissue. It is this hyphal mat which forms the membrane surrounding the sorus (Fig. 3). A column of pistil tissue is left intact at the center of the sorus from the apex to the base. This column of tissue, presumably stimulated by the fungus, elongates with the sorus and maintains contact with the style that is forced out of

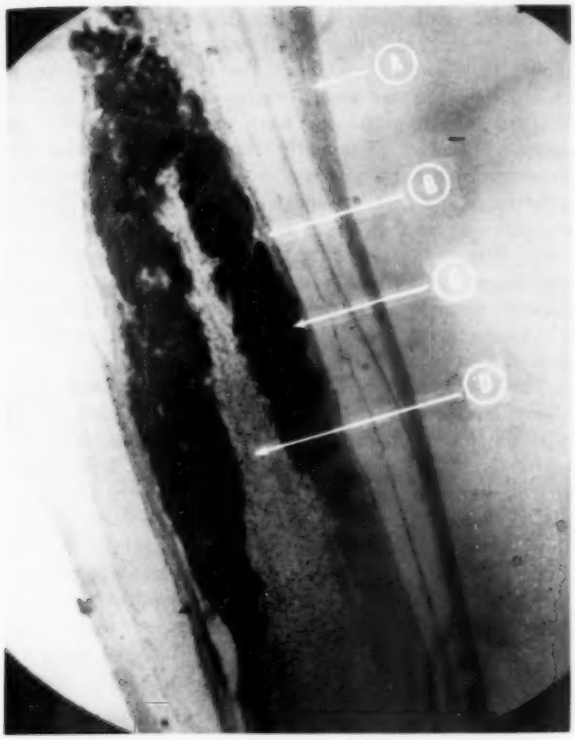

FIG. 3. Longisection of a sorus of *S. occidentalis*. ×100. A) Lemma. B) Outer membrane of fungus tissue. C) Chlamydospores. D) Columella.

the flower proper and may be found at the apex of a fully developed sorus.

A. gerardi develops both sessile and pedicellate flowers. The pedicellate flowers are staminate whereas the sessile flowers are perfect. Hansing (3) found that only the sessile flowers were smutted by *S. occidentalis*.

Cultural studies.—Good growth of the fungus was obtained on the following agar media: potato-dextrose, cornmeal-dextrose, Raulin's, and Richard's (8). The fungus was also grown on moist sterilized greenhouse soil rich in manure. When colony diameter was used as an index of growth, the optimum temperature for growth of the various monosporidial lines varied from 25° to 33°C. The optimum pH for growth for all cultures was pH 6.0. The optimum temperature for chlamydospore germination was between 26° and 28°C.

The chlamydospores of this fungus usually germinated by means of from 1 to a few germ tubes that branched rapidly and produced sporidia in large numbers within 48 hours. The fungus rarely germinated by means of a 1- to 4-celled promycelium that produced primary sporidia. The sporidia budded in profusion, producing large numbers of secondary sporidia.

Three chlamydospore collections were used in this study. One collection was made 4 miles north of Lincoln, Nebraska, and was designated as the U collection. The second collection was made 7 miles west of Lincoln, and was designated as the A collection. The third collection was made in the Dewey Lake region, 20 miles south of Valentine, Nebraska, and was designated as the D collection. Two series of monosporidial isolates were made.

In the first series, 5 monosporidial isolates were obtained from sporidia produced by a single chlamydospore from the A collection. An equal number of isolates was similarly obtained from the D and U collections. In the second series of isolations, single sporidia from each of 5 spores from a single sorus were isolated. This procedure was repeated for each of the 3 chlamydospore collections.

The object in isolating the monosporidial lines in the manner described was to determine any cultural differences between monosporidial isolates from a single chlamydospore as compared to monosporidial isolates from a number of spores from the same sorus.

When all monosporidial isolates were compared, all of the D isolates were similar, as were all of the A isolates. In the U isolates, however, there was a decided difference in cultural characters. All of the first series of U isolates were of the A type, but the second series of U isolates was of a different and distinct type with the exception of an isolate designated as U-2-5. This isolate also was of the A type. Mutant forms similar to the distinct type of U isolates were observed occasionally throughout the study.

Chlamydospores from each of the 3 collections were stored at room temperature for 8 months after which they were tested for the time required for germination on various agar media. Each collection of chlamydospores was tested in triplicate on each medium. Two hundred single chlamydospores were counted in each petri dish, and the number of germinated chlamydospores recorded at the end of 24, 48, and 120 hours. The percentage germination was determined, and the 3 replications were averaged.

Chlamydospores from the D collection germinated promptly and had a uniformly high percentage of germination except in distilled water. Chlamydospores from the A and D collections germinated more slowly and usually had much lower percentages of germination. The highest germination in each of the chlamydospore collections occurred on different media. In the D collection, potato-dextrose agar gave the best germination; in the A collection, Richard's agar; and in the U collection, bluestem-dextrose agar gave the highest germination. The poorest germination in all chlamydospore collections occurred in distilled water. In no case did more than 25 per cent of the chlamydospores germinate in distilled water in less than 120 hours. The D collection of chlamydospores gave at least 60 per cent germination on all agar media tested within 24 hours. The U collection gave at least 25 per cent germination on only 2 media during the same period.

Mutations appeared as sectors in isolates rather frequently. Thirteen mutants were isolated and grown for at least 5 months. All mutant types maintained their characteristics; however, some mutants mutated a second time. In no case did a mutant isolate revert to its original form. Mutant isolates varied from the

parent either in cultural characteristics, rate of growth, or both.

Discussion.—The gradual increase in degree of stunting and consequent death of smutted plants probably can be traced to the gradual ramification of fungus hyphae throughout the rhizome. The direct cause of the death of plants during the induction of dormancy is unknown; however, 1 possible explanation might be that extremely poor root development of the plants in advanced stages of stunting undoubtedly results in low storage of nutrient reserves in the rhizome. The accumulation of metabolic products secreted by the fungus could conceivably contribute to the deterioration and ultimate death of the already weakened plant.

Infection studies indicate that primary infection occurs in the rhizomes of the plant. Since it was found that the fungus is capable of growing in moist soil rich in organic matter, it would be only logical to assume that spores falling to the ground could germinate and grow saprophytically for a short time before reaching the rhizomes. The exact portion of the rhizome where primary infection occurred was not established. It has been commonly stated in the literature that a number of smut fungi infect tender young shoots as they force their way to the surface through the soil; however, no infection was obtained when shoot buds and very young shoots were inoculated, or when soil in a bluestem pasture was seeded with chlamydospores.

The reason why a larger percentage infection was not obtained from inoculated plants is not clear. Environmental conditions in the greenhouse may have been more unfavorable than those in the field for infection to take place. The host plants showed a very high degree of intraspecific variation that may have been responsible for the presence of resistant and susceptible types of plants. This may also explain in part why the smut occurs only in relatively small isolated patches in bluestem pastures.

Cytological studies revealed that the fungus hyphae invaded host parenchyma cells along the plant axis from the rhizome to flower parts of mature plants. The sudden presence of rather large hyphae in the pistils of sessile flowers at the time of chlamydospore formation suggests that small segments of hyphae become distributed throughout the cells at the time of cell division in the meristem tissues, despite the fact that no such hyphal cells were positively identified in meristem tissue. The only other explanation for the presence of the hyphae at this time would be that they penetrated the pistil tissue from cell to cell, following the advance of the apical meristem. No such hyphae were observed. The only typical hyphae observed in pistils in which chlamydospores were not being formed were in pedicellate flowers, and here the hyphae were found only in the vicinity of the base of the flower.

The pattern in which chlamydospore formation takes place immediately suggests that cell division might play an important role in distribution of the fungus in pistil tissue. There is a central area of host tissue surrounded by a comparatively thick covering of fungus tissue in which chlamydospores are formed, and this layer in turn is ensheathed in a relatively thin cover of host tissue whose cells are infected with fungus hyphae that do not form chlamydospores. A more detailed cytological study with improved stain technique might show a definite relationship between meristematic cell division and the formation of the sorus.

It is interesting to note that the symptoms of *Sorghum halepense* (Johnson grass) infected with *Sphacelotheca holci* as described by Johnson et al (4) are similar to the symptoms expressed by *A. gerardi* when infected with *S. occidentalis*. The dwarfing of Johnson grass is often so severe that smutted panicles are produced on weak tillers only a few inches tall. Smutted panicles of Johnson grass also appear earlier than do the healthy panicles. Leukel and Martin (7) have reported that inoculated stubble of Johnson grass produces smutted plants and that the mycelium spreads throughout the crowns and rhizomes of infected plants.

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THE INFLUENCE OF NITRITE ON THE DEVELOPMENT OF PHYTOPHTHORA ROOT ROT OF AVOCADO[1]

George A. Zentmyer and Frank T. Bingham

SUMMARY

Studies were conducted on the influence of nitrite 1) on the development of Phytophthora root rot of avocado in nutrient solution cultures in the glasshouse, and 2) on mycelial growth and zoospore germination of *Phytophthora cinnamomi* Rands.

The toxicity of nitrite to both the fungus and the avocado plant increased as the acidity of the nutrient solution or agar substrate was increased. This relationship held true for mycelial growth, germination of zoospores, and infection of avocado seedlings. Approximately 10 times as much nitrite was required for retardation of mycelial growth and inhibition of zoospore germination at pH 6.5 as at pH 4.5.

In solution culture tests in the glasshouse, development of Phytophthora root rot of avocado seedlings was prevented by the presence of 2 ppm nitrite nitrogen at pH 4.5; at pH 6.5, 40 ppm nitrite nitrogen was required for inhibition of infection.

No evidence was obtained in these experiments that nitrite predisposes avocado roots to infection by *P. cinnamomi;* root injury caused by nitrite did not increase the rapidity or severity of root attack by the fungus. The fungus was more sensitive to nitrite than was the avocado plant; this suggests that under certain conditions nitrite might retard disease development.

Excess soil moisture is apparently an important factor in the disease syndrome, primarily because it provides a medium that the fungus requires for formation of sporangia and for liberation and germination of zoospores.

Phytophthora root rot of avocado, caused by the soil fungus *Phytophthora cinnamomi* Rands, has been reported by a number of investigators to be associated with excess soil moisture. The first report of this disease on avocado trees by Tucker in 1929 (12) noted that "The disease occurs frequently in heavy soils and in poorly drained locations." The reference by Horne et al (8), in 1923, to water injury to the avocado in California and to the sensitivity of this plant to poor drainage probably refers to the same disease, although the causal organism was not reported from California until 1942 (13). A number of other references note the necessity for excess soil moisture in the development of this disease on avocado trees as well as on other hosts (3, 7, 15, 16). Wager (13) reported the necessity for partial submergence of potted avocado seedlings for 24 hours or more in order for *P. cinnamomi* to injure the roots. He also found that if plants were not overwatered they could remain in soil infested with *P. cinnamomi* for 6 months without obvious injury.

There are several possible explanations for the relationship between excess soil moisture and this disease. Root injury may be the result of 1) reduction in the oxygen content of soil under "waterlogged conditions;" 2) accumulation of toxic products such as nitrites, sulfides, carbon dioxide, and methane in the soil under conditions of reduced aeration; 3) one or both of the above factors plus the action of saprophytic or facultatively parasitic soil organisms; and 4) the favorable effect of excess soil moisture on the fungus.

Injury to higher plants from lack of oxygen in the soil is a recognized phenomenon, and wet poorly drained soils often have a low oxygen-supplying power (9). Curtis (4) has shown that the avocado tree is more sensitive than the citrus tree to injury resulting from low oxygen content of nutrient solutions. Curtis and Zentmyer (5) presented experimental data from tests in nutrient solution, showing that *P. cinnamomi* attacked avocado roots most rapidly under conditions of good aeration (6.8–7.2 ppm O_2). Reduction of O_2 to a low level (0.05 ppm) retarded disease development but caused some root injury. This indicates that low O_2 is not an important factor in disease development, but that some root injury might be expected if the oxygen content of soil reached a comparable low level for a number of hours. Furr (6) concluded that that the oxygen content of soils in which Phytophthora root rot of avocado occurred rarely reached a point sufficiently low to be injurious.

Klotz and Sokoloff (10, 11) suggested that accumulation of nitrites as well as of other toxic materials took place in water-logged soils and accounted for much of the injury in what was then termed "avocado decline." These workers produced root damage on avocado seedlings with nitrite and demonstrated that *P. cinnamomi* can itself reduce nitrate to nitrite under slightly acid soil conditions. Klotz and Sokoloff (10) also proposed that injury to roots from various toxic materials might render the roots more susceptible to invasion by saprophytic soil fungi.

In diseases caused by species of *Phytophthora* in general, moisture is necessary for formation of sporangia and for the liberation and germination of zoospores. Thus, the beneficial effect of moisture on fungus activity must be considered in the over-all picture of disease development. In reference to *P. cinnamomi* on avocado in Peru, Crandall (2) con-

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cluded that the effect of excess soil moisture was more important to the fungus than to the host. Zentmyer (14) suggested that soil moisture was important primarily because of the necessity for water in the life cycle of the fungus.

In a further investigation of the interrelationships among soil moisture, various toxic materials, and the causal fungus, the influence of nitrite on the development of Phytophthora root rot was examined. Results are reported in this paper.

MATERIALS AND METHODS.—The effect of nitrite on avocado seedlings and on the development of Phytophthora root rot was investigated in the glasshouse by use of aerated nutrient solutions in 100-liter glazed ceramic cylinders. The experimental apparatus was described in a recent article by Bingham and Zentmyer (1). The nutrient solution contained all elements necessary for normal growth of the avocado. Solutions were prepared by adding $Ca(NO_3)_2$, $MgSO_4$, and KH_2PO_4, respectively, at the rate of 2.5, 1.0, and 1.0 millimoles per liter of culture solution; in addition minor elements were supplied in the following concentrations: 0.25 ppm B, 0.25 ppm Mn, 0.03 ppm Zn, 0.01 ppm Cu, 0.005 ppm Mo, and 1.0 ppm Fe (as $FeSO_4$).

The effect of nitrite was studied at pH 4.5 and pH 6.5 because the acidity of the nutrient solution has a marked effect on the toxicity of nitrite (the more acid the solution, the more toxic a fixed concentration of nitrite becomes). The reaction was adjusted to the desired level and maintained within 1/10 of a pH unit by means of H_2SO_4 or KOH. Nitrite was added in the form of potassium nitrite in concentrations calculated to give the desired levels of NO_2-N.

Mexican avocado seeds (Mexicola variety) were germinated in steam-sterilized sand, and the seedlings were transferred to the nutrient solution after reaching a height of 1–3 in. Three seedlings were placed in each 100-liter cylinder. After the seedlings had grown several weeks in the nutrient solutions at either pH 4.5 or pH 6.5, the nitrite additions were made, and the inoculum was placed in the solutions.

Inoculum was prepared from fungus grown on packets of sterilized alfalfa stems in test tubes. After 2 weeks' growth of the fungus on the stems, the packets of 4–5 stems were wrapped in gauze. These bags were then suspended from the lids of the culture solution apparatus so that they were submerged and in the path of the circulating nutrient solution.

In laboratory tests with *P. cinnamomi*, amounts of sodium nitrite solutions calculated to give the desired concentrations of NO_2-N were pipetted under aseptic conditions into flasks containing 45 ml of melted cornmeal agar adjusted to either pH 4.5 or pH 6.5. Triplicate plates were then poured from each flask. The plates were seeded in the center with a disk 5 mm in diameter from a culture of *P. cinnamomi*, and colony diameter was measured at intervals during incubation at 25°C.

The effect of nitrite on germination of zoospores of *P. cinnamomi* was studied in glass depression slides. Zoospores were obtained from sporangia formed on avocado roots that were invaded by *P. cinnamomi* in nutrient solution cultures. Zoospore discharge took place in 15–30 minutes after the roots were placed in tap water in open Petri dishes at room temperature (24–27°C). Richards' solution adjusted to either pH 4.5 or 6.5 and containing NO_2-N at twice the final concentration desired was pipetted into the depression slides. Equal amounts of zoospore suspension were then pipetted into each slide to give the desired concentration of NO_2-N, and the slides were incubated for 4 hours at room temperature. Zoospores began to germinate within 1 hour after their release from sporangia.

RESULTS.—*Development of root rot.*—The effect of nitrite on development of Phytophthora root rot of avocado seedlings in solution culture at pH 4.5 was studied in 3 experiments. In the first of these, 36 seedlings were grown in a total of 12 cylinders divided into 3 sets of 4 cylinders each. The first set received a conventional nutrient solution; the second set received, in addition to the nutrient solution, a 5-day pretreatment of 2 ppm NO_2-N before being seeded with *P. cinnamomi;* and the third set was supplied continuously with nutrient solution plus 2 ppm NO_2N. Half of the seedlings of each set were inoculated.

Within 3 days after inoculation, typical brown Phytophthora lesions appeared on roots in all treatments except that in which 2 ppm NO_2-N was maintained. Nine days after inoculation, root systems were completely rotted and foliage was severely wilted in plants growing in the conventional nutrient solution and in those receiving the 2 ppm NO_2-N pretreatment. Plants receiving the continuous treatment of 2 ppm NO_2-N did not develop root rot (Table 1).

Progress of disease in plants receiving the nitrite pretreatment was similar to that in plants growing in conventional nutrient solution, indicating that pretreatment with NO_2-N did not predispose roots to infection. This series of treatments was repeated in a second experiment, and identical results were obtained.

In the third experiment at pH 4.5, the possibility that nitrite might predispose avocado roots to invasion by *P. cinnamomi* was further investigated. The treatments were 1) complete nutrient solution alone, 2) complete nutrient solution plus 2 ppm NO_2-N for 5 days prior to inoculation with the fungus, and 3) complete nutrient solution plus 24-hour pretreatment with 4 ppm NO_2-N prior to inoculation. Each treatment involved 12 Mexicola avocado seedlings, with 3 seedlings in each of 4 ceramic cylinders. At the end of the pretreatment period, the seedlings were transferred to other cylinders containing the nutrient solution alone.

In this experiment the nitrite pretreatments caused some injury to the small feeder roots. The injury consisted primarily of brown lesions at the root tips. Based on visual examination of the roots, this restricted the amount of root tissue that could be invaded by *P. cinnamomi*.

No acceleration of root rot or of wilting of the

TABLE 1.—*Phytophthora root rot and root injury in avocado seedlings grown in solution cultures adjusted to pH 4.5 and containing no NO_2-N, containing NO_2-N for 5 days prior to seeding with P. cinnamoni, and containing NO_2-N throughout the experiment*

Treatment	Solution seeded with *P. cinnamomi*[a]	Percentage of root rot or root injury[b] after 4 days	6 days	9 days	30 days
(Experiment 1)					
Nutrient alone	−	0	0	0	0
	+	33	85	99	100
2 ppm NO_2-N for 5 days prior to seeding	−	2[c]	2	2	2
	+	42	87	100	100
2 ppm NO_2-N supplied before and after seeding	−	3[c]	3	3	3
	+	3[c]	3	3	3

Treatment	Solution seeded with *P. cinnamomi*	2 days	3 days	4 days	5 days	8 days
(Experiment 2)						
Nutrient alone	+	0	12	39	63	93
2 ppm NO_2-N for 5 days prior to seeding	+	27[c] (0)	41 (14)	67 (40)	79 (52)	94 (67)
4 ppm NO_2-N for 1 day prior to seeding	+	31[c] (0)	40 (9)	56 (25)	75 (44)	91 (60)

[a] A gauze bag containing inoculum of *P. cinnamomi* grown on sterilized alfalfa stems was suspended in the nutrient solution.

[b] Root rot and root injury were determined by visual examination of roots and estimation of percentage of roots affected. Figures in parentheses are root rot percentages corrected for initial injury resulting from nitrite. Figures are averages of data from 9–12 plants.

[c] Root injury caused by nitrite.

foliage occurred as a result of the nitrite pretreatments (Table 1). When the initial figures for root injury caused solely by nitrite are subtracted from the total figures for root damage on the inoculated plants, it appears that the plants pretreated with nitrite had less root rot than the controls. It could not be determined by examination of the roots whether or not the lesions produced by nitrite injury also were later invaded by *P. cinnamomi*. This point could be determined only by culturing all lesions, and this was not possible inasmuch as there were several thousand lesions in each culture. It is evident from the data, however, that the nitrite pretreatment did not accelerate disease development.

In the experiments at pH 6.5, seedlings were grown in nutrient solutions containing 0, 5, 10, 15, 20, 30, 40, and 50 ppm NO_2-N, and half of the seedlings of each set were inoculated. The retarding effect of nitrite on Phytophthora root rot was also evident in these tests. Inhibition of disease development took place at 40 and 50 ppm NO_2-N at pH 6.5, in contrast to inhibition at 2 ppm at pH 4.5 in the preceding tests. Results of the tests are shown in Fig. 1, where disease development at each concentration of NO_2-N is plotted as an individual curve.

At pH 6.5, 50 ppm NO_2-N caused little or no injury to the avocado roots. At pH 4.5, 5 ppm NO_2-N caused severe root damage, and 2 ppm NO_2-N resulted in some damage to the root tips.

Toxicity of nitrite to the fungus.—Results of agar plate tests also showed the sensitivity of *P. cinnamomi* to small amounts of nitrite. A higher concentration of nitrite was required for growth inhibition at pH 6.5 than at pH 4.5.

At pH 4.5, the fungitoxic concentration of nitrite was 6 ppm NO_2-N; some reduction in growth was obtained at 4 ppm (Table 2). At pH 6.5, some extremely weak and sparse growth of the fungus took place at 45 and 60 ppm NO_2-N (Table 2); there was no normal fungus growth when the concentration of NO_2-N was above 30 ppm. Thus approximately 10 times as much nitrite was required for significant retardation of mycelial growth of *P. cinnamomi* at pH 6.5 as was required at pH 4.5.

At pH 4.5, germination of *P. cinnamomi* zoospores was significantly reduced by nitrite concentrations greater than 4 ppm (Fig. 2). Only 2 per cent germination occurred at 16 ppm NO_2-N, and there was no germination at 32 ppm.

At pH 6.5, a much higher concentration of nitrite was required for retardation and inhibition of germination. Zoospore germination was significantly re-

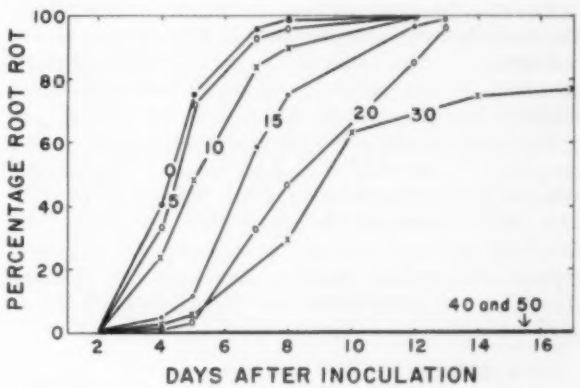

FIG. 1. Progress of Phytophthora root rot of avocado in nutrient solution at pH 6.5, in relation to concentration of nitrite-nitrogen maintained (0, 5, 10, 15, 20, 30, 40, and 50 ppm).

TABLE 2.—*Growth of colonies of Phytophthora cinnamomi on cornmeal agar adjusted to various pH levels and containing different amounts of NO_2-N*

pH	NO_2-N (ppm)	Diameter of colonies (mm) after[a] 3 days	5 days	7 days
4.5	0	47	69	89
4.5	2	43	66	88
4.5	4	24	47	75
4.5	6	0	0	0
6.5	0	41	59	90
6.5	15	21	35	79
6.5	30	14	27	56
6.5	45	0	10	14
6.5	60	0	7	12

[a] Each figure represents an average of 3 replications.

duced at 80 ppm NO_2-N. For complete inhibition (Fig. 2), 320 ppm NO_2-N was required.

DISCUSSION.—It is evident from the data presented that *P. cinnamomi* and the avocado plant respond similarly to the nitrite-pH relation. The toxicity of nitrite to both the fungus and the plant increases as the acidity of the nutrient solution or substrate is increased. This relationship applied to mycelial growth of the fungus, to germination of zoospores, and to infection of the avocado plant.

On the basis of these experiments it seems unlikely that nitrite would predispose avocado trees to infection by *P. cinnamomi*. Pretreatment of avocado seedlings with 2 ppm and 4 ppm of NO_2-N prior to inoculation with *P. cinnamomi* did not accelerate disease development significantly over that on plants not subjected to such pretreatment. Plants injured by nitrite pretreatment were not more severely nor more rapidly attacked by *P. cinnamomi* than uninjured plants. The data on inhibition of infection by nitrite suggest that nitrite in the soil could be either fungistatic or fungicidal to *P. cinnamomi*, thus retarding the development of the root rot. The fungus is evidently more sensitive to injury from nitrite than is the avocado, as concentrations of NO_2-N that prevented root invasion did not injure the avocado seedlings.

The results presented here provide a further contribution toward an understanding of the role that excess soil moisture plays in the development of Phytophthora root rot. They suggest that injury to roots from toxic materials such as nitrite may not be of general significance in disease development. Earlier work on the relation of reduction in oxygen level to disease development (5) indicated that this factor, also, probably is not important. It appears that, primarily, excess soil moisture provides a medium in which this type of fungus may produce sporangia and liberate zoospores, and in which the zoospores may swim about, germinate, and infect roots.

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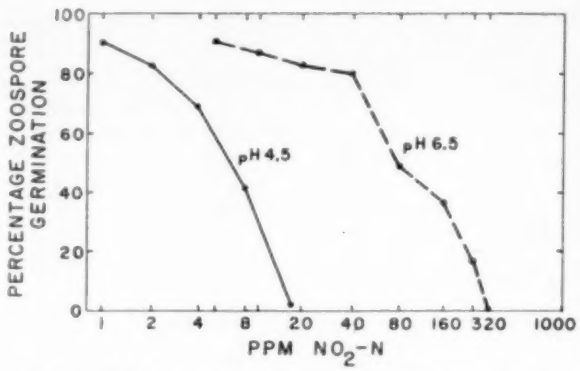

FIG. 2. Effect of nitrite-nitrogen concentration on germination of zoospores of *Phytophthora cinnamomi* in nutrient solution at pH 4.5 and pH 6.5. (Plotted on semilogarithmic scale.)

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ALTERING DISEASE RESISTANCE WITH IONIZING RADIATION [1]

Paul E. Waggoner and A. E. Dimond

SUMMARY

Radiation both increased and decreased resistance of 5- to 6-week-old Bonny Best tomato plants to Fusarium wilt. Whole-plant irradiation before inoculation increased resistance, whereas the same treatment at inoculation time decreased resistance. Root irradiation before inoculation increased resistance significantly, but the same treatment at inoculation time caused only a small increase. Shoot irradiation at any time decreased resistance, but not significantly.

Vascular wilts, such as Dutch elm disease, oak wilt, flax wilt, and various Fusarium and Verticillium wilts cause great economic losses. These diseases have proved difficult to control; consequently, pathologists have been forced to develop novel control measures such as chemotherapy (4). In addition, tests of therapy by ionizing radiation have been initiated (9, 10).

Fusarium wilt of tomato (*Lycopersicon esculentum* Mill.) was chosen for radiation studies because of the information already available concerning its physiology (3, 6) and chemotherapy (4) and because of convenience. The present report concerns the alteration by X-rays of the resistance of the tomato to this wilt.

MATERIALS AND METHODS. — Bonny Best tomato plants were grown at 27°C in sand in greenhouse benches with a constant water level. They received nutrients 3 times a week (4). Five- to 6-week-old plants having 4–6 true leaves were used.

All irradiation was performed with a Coolidge X-ray tube operated at 6.2 Kv and 5 mamp. A 33 mg/cm^2 aluminum filter, which was placed between target and plant, resulted in an intensity of 5 r/sec. at 15 cm from the target.

Plants to be exposed to radiation were uprooted and laid upon a pad of wet cotton beneath the tube. Control plants were exposed in the adjacent room in the same manner.

Plants to be inoculated were uprooted, and the wounded roots were dipped in a suspension of bud cells from shake culture of strain R5-6 of *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans. in casein hydrolysate medium (5). The plants were repotted, then incubated about 14 days at 27°. The severity of necrosis, yellowing, and wilting (loss of turgor) of foliage was graded on a scale from 0 to 4. The internodes were cut, and the mean number of primary bundles discolored per internode was determined for each of 5 plants. This value, called the vascular index, can range from 0 to 6. These methods are essentially the same as those used (4) in the Fusarium wilt assay of chemotherapeutants.

WHOLE PLANT IRRADIATION AT INOCULATION TIME.—Field experiments made previously suggested that plants exposed to chronic irradiation following inoculation were more susceptible to wilt than were nonirradiated ones (10). Five experiments under the more easily controlled conditions of a greenhouse were run to test this possibility. The entire plant was subjected to radiation; inoculation followed immediately. Radiation increased the severity of both foliar (Fig. 1) and vascular (Fig. 2) symptoms in about the same way in these experiments, which were performed in all seasons of the year. In other words, radiation in this manner decreased resistance.

WHOLE PLANT IRRADIATION BEFORE INOCULATION.—Growth regulators increase resistance more when treatment precedes inoculation than when the 2 are simultaneous (2). It appeared possible that radiation might have a similar effect. To test this hypothesis, whole plants were exposed to 21 Kr of X-rays, 10, 5, and 0 days before inoculation. Appropriate checks were provided by nonirradiated plants that were uprooted and taken to the X-ray room at the same time as the irradiated ones. Vascular discoloration in plants irradiated before inoculation was less, and that in plants irradiated at inoculation time was greater, than that in the corresponding checks (Table 1). That is, irradiation before inoculation increased resistance, and irradiation at time of inoculation increased susceptibility. Unfortunately, the high dosages used caused undesirable stunting.

LOCALIZED IRRADIATION.—Many workers, beginning with May (7), have shown that the root and shoot of the host make different contributions to resistance. This suggested that resistance of root and shoot might be affected differently by radiation and that a method that increased resistance without stunting could be obtained.

Plants were uprooted 5 days before, at the time of, and 5 days after inoculation. At each time, some en-

TABLE 1.—*Vascular index in tomato plants irradiated at different intervals prior to inoculation*

Radiation dose (Kr)	Vascular index[a] in plants irradiated at the indicated intervals prior to inoculation		
	10 days	5 days	0 days
0	1.6	0.9	1.1
21	0.5	0.0	1.8

[a] LSD (P = 0.05) = 0.9.

[1] Accepted for publication September 16, 1955.

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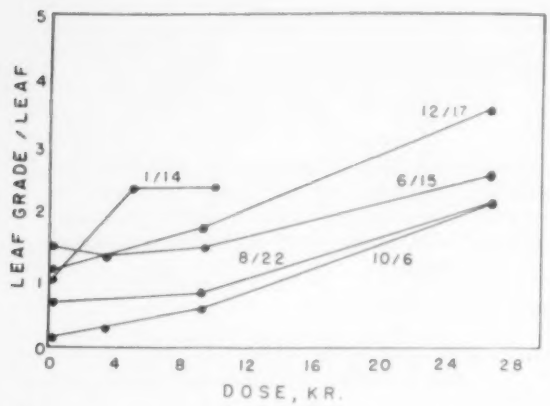

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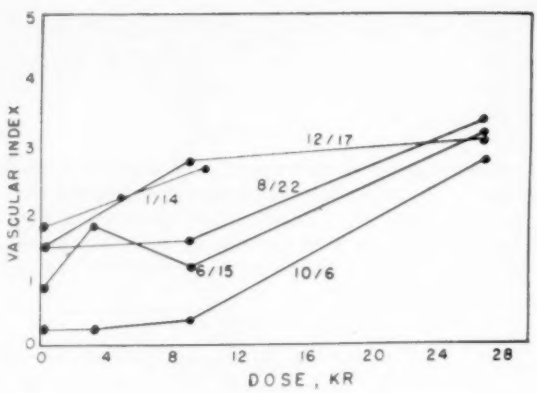

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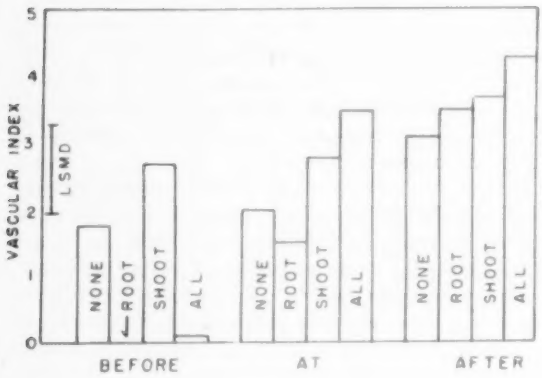

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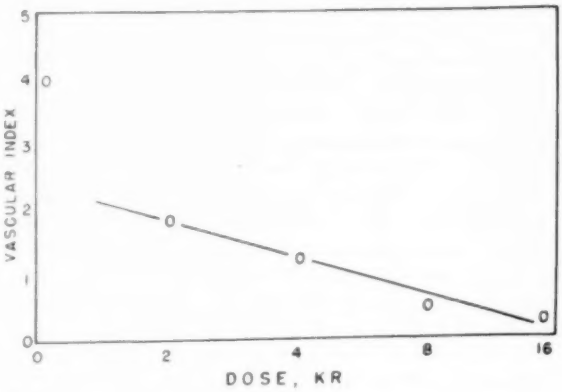

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FIG. 1-4.—Fig. 1-2. Severity of leaf and vascular symptoms of Fusarium wilt following whole-plant irradiation at inoculation time.—Fig. 3. Vascular indices due to Fusarium wilt following localized irradiation at 3-times relative to inoculation.—Fig. 4. Vascular indices due to Fusarium wilt following root irradiation 5 days before inoculation.

tire plants were exposed to 18 Kr, others received 18 Kr in the shoots, others received 18 Kr in the roots, and others received none. All plants were inoculated at the same time and were arranged, after repotting, in split plots with "times" for whole plots.

As expected, whole-plant irradiation before inoculation increased resistance, whereas the same treatment at or after inoculation decreased resistance relative to the checks (Fig. 3). Root irradiation before inoculation significantly increased resistance, whereas this treatment at inoculation time increased resistance only slightly. Shoot irradiation at any time decreased resistance, but not significantly. Any treatment after inoculation decreased resistance, but not significantly. A comparison of nonirradiated plants at the 3 times shows that transplanting before inoculation caused some increase, whereas transplanting after inoculation caused some decrease in resistance.

In a second experiment, plant roots were exposed to varying doses 5 days before inoculation. Between 2 and 16 Kr, doubling the dose decreased vascular discoloration by about 0.6 (Fig. 4). The 2-Kr dose decreased discoloration significantly.

As 2 criteria of the usefulness of root irradiation, in addition to increase in resistance, plant height and root weight were determined at the time of disease observation. The mean plant heights for 0, 2, 4, 8, and 16 Kr were 6.3, 8.1, 5.6, 8.1, and 7.9 cm, respectively. The mean root weights were 1.2, 1.6, 1.1, 1.5, and 1.3 g, respectively, for the same doses. These observations show that by proper choice of method, the benefit of radiation—increased resistance—can be greater than the injury from radiation.

DISCUSSION.—X-radiation was used in these studies but there is no reason to believe that another form of ionizing radiation would not accomplish the same quality of results.

A possible explanation for the occasional reports of beneficial effect of radiation upon plants is provided by the experiment summarized in Figure 4 and in the preceding section. With the proper localization and timing of radiation, the plants for 3 out of 4 doses were larger than the checks. This occurred only because of the presence of a pathogen to which the

plants had been rendered resistant. Nevertheless, in this environment the results showed radiation to be beneficial. Possibly some observations of benefit from radiation have been due to similar circumstances.

The many studies of the alteration of auxin level by radiation following Skoog's (8) early work and of the chemotherapeutic action of growth regulators (2) already have led to the suggestion that auxin regulates resistance and that radiation alters resistance by altering auxin levels (10). Success in the present studies in predicting the response to radiation from known response to growth regulators increases our interest in that suggestion. This suggestion is also consistent with the observation that root exposure and shoot exposure evoke different responses, because the classical view is that root and shoot play differing roles in auxin synthesis and use or destruction (1). Although these suggestions certainly have not been proved here, they provide a basis for further studies on the physiology of wilt and on the effect of chemicals and radiation on resistance.

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PHYTOPATHOLOGICAL NOTES

Probable Coidentity of Shiro Line Pattern Virus and Apple Mosaic Virus. R. M. Gilmer. Cochran reported symptoms similar to those of apple mosaic in apple seedlings bud-inoculated from peach containing ring spot virus.[1] A recent report by Kirkpatrick[2] showed that apple mosaic virus uncontaminated with ring spot virus was transmissible from apple to peach and from peach to apple. His illustration of the symptoms of apple mosaic in peach are strikingly like those incited by Shiro line pattern virus in the same host.

In 1950, during initial attempts to transmit necrotic ring spot virus from sour cherry to apple, bud inoculations were made to apple seedlings from 8 sour cherry trees infected with necrotic ring spot virus. Certain of the inoculum sources were known to contain sour cherry yellows virus.

Symptoms indistinguishable from those of apple mosaic occurred in 3 of 6 apple seedlings inoculated with isolate A10/1-4 (recurrent necrotic ring spot and sour cherry yellows viruses), but none of the remaining isolates produced mosaic symptoms in apple although some incited small depressed necrotic rings in the leaves of some seedlings.[3] The actual identity of the virus from isolate A10/1-4 that incited mosaic symptoms in apple could not be determined from the available data, but the fact that other cherry isolates carrying necrotic ring spot and sour cherry yellows viruses failed to incite mosaic symptoms in inoculated apple seedlings appeared to exclude these 2 viruses from consideration as incitants of the mosaic syndrome in apple.

In 1952, Parker[4] showed by field inoculation of Shiro plum that line pattern virus was present in cherry isolate A10/1-4 in addition to recurrent necrotic ring spot and sour cherry yellows viruses. This evidence raised the possibility that the mosaic symptom in apple might actually have been incited by line pattern virus.

A series of apple seedlings and a series of Shiro plum budlings were bud-inoculated in July, 1953, with isolate A10/1-4 obtained from plum. Two additional series of apple and Shiro plum trees were bud-inocu-

[1] Cochran, L. C. 1950. Infection of apple and rose with ring-spot virus. (Abs.) Phytopathology 40: 964.
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[4] Parker, K. G. Unpublished data.

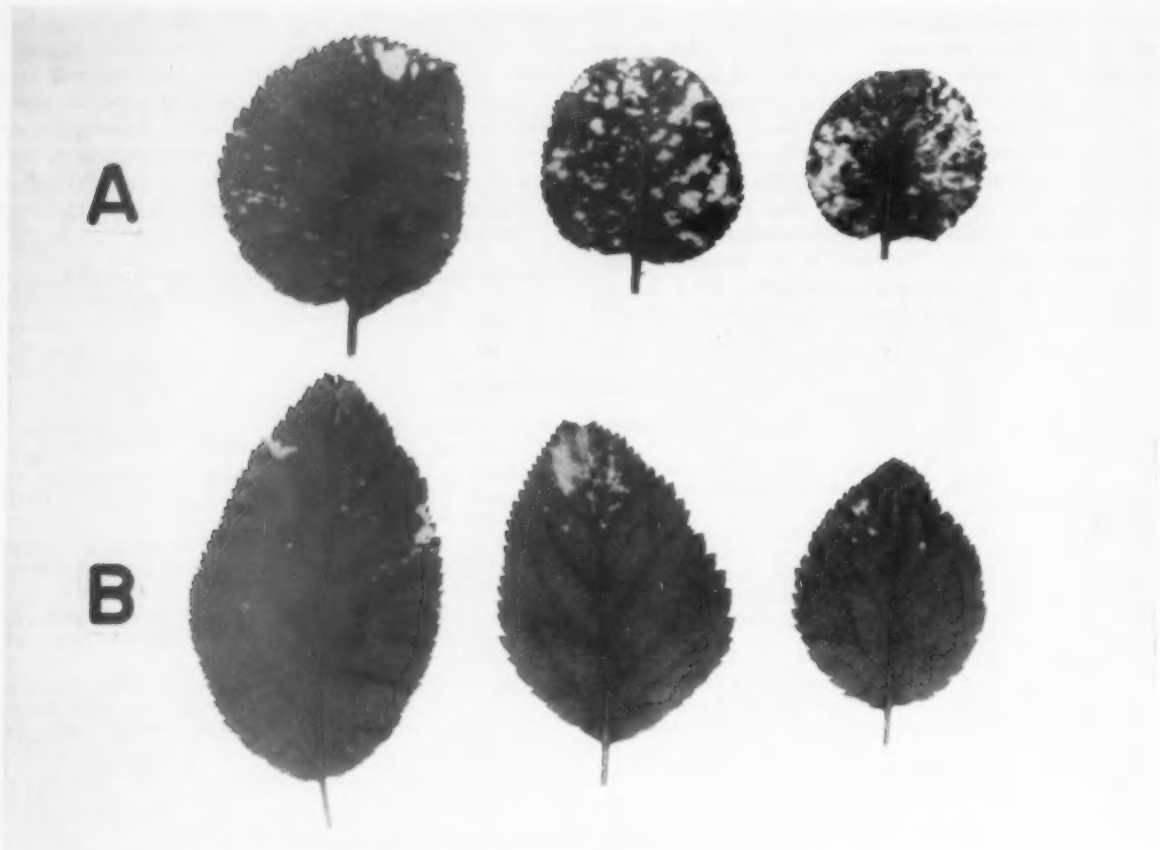

FIG. 1. The effect of temperature on expression of symptoms of line pattern virus in apple seedlings. A) Alternating temperatures of 75°F (days) and 55°F (nights). B) Constant temperature of 75°F.

TABLE 1.—*Symptom expression of reciprocal inoculations of line pattern virus and apple mosaic virus in seedling apple and Shiro plum*

Virus	Isolate No.	Source of inoculum	Reaction in apple[a]	Reaction in Shiro[a]
Apple mosaic	51A1-1	Apple	AM (8/8)	LP (5/8)
Line pattern	A10/1-4	Plum	AM (3/8)	LP (7/8)

[a] AM indicates apple mosaic symptoms; LP, line pattern symptoms. Numerator of numbers in parenthesis denotes number of trees infected; denominator, number of trees inoculated.

lated directly from apple infected with an apple mosaic virus originally obtained from a mature orchard tree in Wayne County, New York.[5] The inoculated trees were forced in the greenhouse in February, 1954, half of the trees of each of the 4 series being grown at a temperature of 75°F, and the remainder at alternating temperatures of 75°F (days) and 55°F (nights).

Each isolate incited symptoms of apple mosaic in apple seedlings and of line pattern in Shiro plum budlings (Table 1). Symptom expression occurred at 75° and at the alternating 75–55° temperatures, but development of chlorotic or creamy-white areas in leaves of affected plants was more pronounced at the alternating temperatures (Fig. 1). With increasing greenhouse temperatures as the season progressed, the occurrence of symptoms in newly formed leaves became increasingly masked in both apple and Shiro plum in the case of both virus isolates. Leaves produced when greenhouse temperatures exceeded 90°F were entirely symptomless in both apple and Shiro plum.

Reisolation of line pattern virus from apple to Shiro plum and of apple mosaic virus from Shiro plum to apple resulted in the production of characteristic symptoms of each virus disease in its respective determinative host.

In view of the fact that New York isolates containing only necrotic ring spot virus when bud-inoculated to apple seedlings incite symptoms consisting of small necrotic rings and that such isolates have not incited symptoms of apple mosaic suggests that Cochran's[1] ring spot isolates from peach may have been carrying line pattern virus. The present data, and those of Kirkpatrick,[2] strongly suggest that apple mosaic virus and line pattern virus of various *Prunus* species are identical.—Department of Plant Pathology, New York State Agricultural Experiment Station, Geneva, New York.

[5] The original isolation was made by Dr. K. G. Parker.

The Effect of the Tobacco Stunt Nematode on the Incidence of Fusarium Wilt in Flue-cured Tobacco.[1] QUINTIN L. HOLDEMAN. When Fusarium wilt, caused by *Fusarium oxysporum* (Schlecht.) Wr. var. *nicotianae* J. Johnson, of flue-cured tobacco is first observed on farms in South Carolina, its future course of destruction cannot be predicted. In some fields, it has been present for many years but has never become important, while in others it has caused serious losses. Limited observations in 1952 and 1953 revealed that plant parasitic nematodes were frequently, but not always, present in soils where Fusarium wilt was severe. In 2 fields observed in 1952, near Mullins and Timmonsville, S. C., the tobacco stunt nematode, *Tylenchorhynchus claytoni* Steiner, 1933, composed over 99 per cent of the parasitic types recovered. These observations, coupled with reports that certain other nematodes influenced the incidence of soil-borne diseases,[2,3,4] indicated a need for a study of the possible influence of the tobacco stunt nematode on the incidence of Fusarium wilt in tobacco.

To establish nematode cultures containing a single parasitic species, nematodes were obtained from field soil by means of Christie and Perry's technique.[5] Individuals of *T. claytoni* were transferred to a drop of water on a slide for confirmation of identity under the microscope. One hundred nematodes of this species were transferred to each of several crocks containing autoclaved soil and planted with cotton or a combination of cotton and tobacco. Roots of cotton and tobacco had been found to serve as a satisfactory medium for increasing populations of this nematode species. When sufficient nematode populations had been obtained, experiments were initiated on tobacco.

Three greenhouse experiments were conducted in which inoculum of the Fusarium wilt fungus was used alone and in combination with the tobacco stunt nematode. Pertinent data pertaining to types of inocula used in each experiment are presented in Table 1. In all cases, inocula were added to crocks partially filled with autoclaved soil. Approximately 3 additional inches of soil were added before tobacco seedlings were transplanted. Five test plants of a wilt-susceptible tobacco variety, Oxford 1-181, were set in each crock but were thinned to 3 after they had become established.

In each experiment, a greater percentage of the plants had external symptoms of wilt than when either or both kinds of inoculum were omitted (Table 1). Thus it is evident that the tobacco stunt nematode is capable of increasing the incidence of wilt in the susceptible tobacco variety, Oxford 1-181. It is probable that this nematode may be one of the factors responsible for the erratic behavior of Fusarium wilt of tobacco in the field.—Pee Dee Experiment Station, Florence, South Carolina.

[1] Technical contribution No. 232, South Carolina Agricultural Experiment Station in cooperation with Section of Tobacco, Medicinal, and Special Crops, Agricultural Research Service, U. S. Department of Agriculture. A contribution to the program of work under Southern Regional Project S-19, Plant Parasitic Nematodes.

[2] Holdeman, Q. L., and T. W. Graham. 1954. The effect of the sting nematode on the expression of Fusarium wilt in cotton. Phytopathology 44: 683-685.

[3] Lucas, G. B., J. N. Sasser, and A. Kelman. 1954. The effect of root-knot nematodes on the expression of Granville wilt resistance in tobacco. (Abs.) Phytopathology 44: 497.

[4] Sasser, J. N., H. R. Powers, Jr., and G. B. Lucas. 1953. The effect of root knot nematodes (Meloidogyne spp.) on the expression of black shank resistance in tobacco. (Abs.) Phytopathology 43: 483.

[5] Christie, J. R., and V. G. Perry. 1951. Removing nematodes from soil. Helminthol Soc. Wash. Proc. 18: 106-108.

TABLE 1.—*Incidence of Fusarium wilt in flue-cured tobacco grown in gallon crocks containing no inoculum, Fusarium, nematodes, or Fusarium plus nematodes*

Experiment	Duration (months)	Number of crocks per treatment	Plants per crock	Fusarium inoculum[a]	Nematode inoculum	Nematodes per crock	Percentage of plants wilted
1	4	8	3	None	None	0	4[b]
				None	Infested soil[c]	460	0
				Liquid culture[d]	None	0	0
				Liquid culture[d]	Infested soil[c]	460	54
2	3	10	3	None	None	0	0
				None	Suspension[e]	1500	0
				Liquid culture[d]	None	0	4
				Liquid culture[d]	Suspension[e]	1500	93
3	3	10	3	Wheat culture[f]	None	0	37
				Wheat culture[f]	Suspension[e]	900	83

[a] Single-spore culture isolated and tested for virulence, courtesy Dr. G. M. Armstrong.

[b] Represents 1 wilted plant and is attributed to contamination by splashing water droplets during watering.

[c] 300 cc infested soil added to each crock.

[d] 300 ml of 7-day liquid culture (1 yeast cake and 1 bouillon cube per liter of water) added to each crock.

[e] 100 ml of a water suspension of tobacco stunt nematode added to each crock.

[f] 1 tablespoonful of 2-week-old wheat grain culture added to each crock.

Transmission to Amelanchier of an Agent Causing a Disorder on Apple[1]. D. F. Millikan and H. W. Guengerich. In the major commercial apple-producing areas of Missouri, the variety Virginia Crab has been recommended as an interstock for orchard trees. Its use has been particularly popular in the northwestern section of the state where late fall temperatures fluctuate greatly. Certain incompatibilities were noted in the first plantings of combinations of Virginia Crab and other varieties and these appear to be increasing. Although this report considers only the abnormality associated with combinations of Virginia Crab and Golden Delicious, other noncongenial combinations have been noted. Miller[2], Smith[3], and Tukey et al[4] list several other combinations that show this same type of symptom.

The abnormality under study usually appears first as a willowy growth in the scaffold branches. Subsequently, superficial lesions vertical to the ground line appear on the Virginia Crab stem piece. Initially, these lesions are mere splits in the periderm about 1–3 in. long and eventually develop into cracks that may be as much as 10 in. long and 1 in. wide. An examination of the xylem tissue invariably reveals a pitting of the tissue likened by Smith[3] to that described by Schneider[5] for the quick decline disease of citrus. That such morphological symptoms suggest an infectious entity to which Virginia Crab is especially sensitive was indicated by Millikan and Guengerich[6] in an earlier report. They observed that diseased apple tissue chip-budded to *Prunus tomentosa* caused a striking mottle on the foliage.

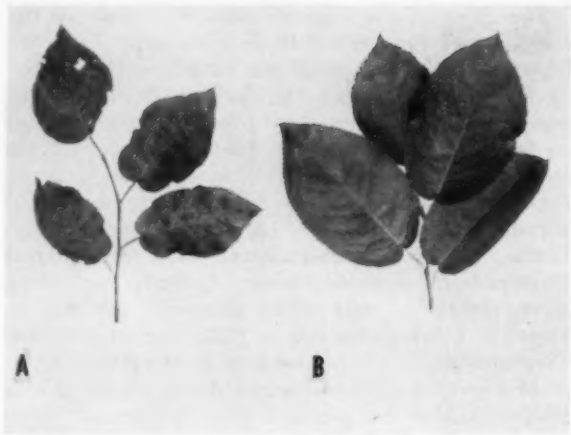

Fig. 1. Symptoms of apple disorder on Amelanchier. A) Diseased spur showing dwarfing, mottling, and rugosity. B) Healthy spur.

On April 7, 1954, buds from a diseased Golden Delicious/Virginia Crab were placed into a shad bush, *Amelanchier* spp., growing in an adjoining wooded area. These buds developed into shoots about 3–4 in. in length, despite the unfavorable dry growing season. No abnormalities were noted until May 10, 1955, when the shad shoot above the inserted apple bud developed striking mottled patterns. The affected leaves were dwarfed and rugose, in sharp contrast with the smooth healthy leaves. The abnormality remained localized, indicating a very slow spread similar to that observed in apple.

This preliminary report suggests that Amelanchier may be a useful indicator plant in apple virus studies. —Department of Horticulture, University of Missouri.

[1] Contribution from Missouri Agricultural Experiment Station, Journal Series No. 1542. Approved by Director.

[2] Miller, V. J. 1955. A trunk disorder of Virginia Crab understock. Amer. Soc. Hort. Sci. Proc. 64: 159-164.

[3] Smith, W. W. 1954. Occurrence of "stem pitting" and necrosis in some body stocks for apple trees. Amer. Soc. Hort. Sci. Proc. 63: 101-113.

[4] Tukey, R. B., R. L. Klackle, and J. A. McClintock. 1954. Observations on the uncongeniality between some scion varieties and Virginia Crab stocks. Amer. Soc. Hort. Sci. Proc. 64: 151-155.

[5] Schneider, H. 1954. Condition of phloem of sour orange tree trunk in winter. Hilgardia 22: 583-591.

[6] Millikan, D. F., and H. W. Guengerich. 1954. Bark splitting, a possible virus disease in apple. (Abs.) Phytopathology 44: 498.